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## STUDIES ON HOST REACTIONS TO LARVAL PARASITES. I. THE EFFECT ON WEIGHT<sup>1</sup>

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### INTRODUCTION

It is realized by most parasitologists that unusually heavy infections by bacteria, molds and fungus, protozoa, worms, glochidia and parasitic arthropods may have a deleterious effect upon the host. The authors, realizing the need of further study of several aspects of this general problem, outlined a series of experiments dealing with host reactions to larval helminths. This paper presents some preliminary results that were obtained.

The first question was whether a heavy infection of larval parasites would have any appreciable effect upon the metabolism of the host. It was decided to look for this effect in a host in the form of some readily measurable criterion such as a difference in weight. While the literature contains many experiments dealing with the effect of parasites upon their host, most fall outside the limits of this paper.

The literature yields several suggestive papers along this line for Hubbs (1927) cites abnormalities in the growth of fish due to heavy infections by trematodes (probably strigeids) and proteocephalid plerocercoids (probably *P. ambloplitis*), and Essex and Hunter (1926) likewise record "horseshoe nail trout," taken in Montana, apparently due to heavy infections of larval tapeworms. Cross (1933, 1934) made a study of fish and their parasites from several Wisconsin lakes. He grouped the hosts according to their age and found differences in size and weight between negative and heavily infected fish of the same age and species.

Strigeid cercariae and related forms constitute the largest group of tissue-penetrating parasites of fresh water fish. Plehn (1924) reports infection of European carp by a strigeid larva called the "pearl holostomum." She states that excessive infection causes a loss of weight but cites no experimental evidence. Van Haitsma (1931) found definite

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pathological symptoms in ducks that were experimentally infected with the strigeid trematode, *Cotylurus flabelliformis*. Krull in 1934 reports infection experiments with the cercaria of *Crassiphiala ambloplitis* (= *C. bessiae*) on centrarchids. He states that the effects depend upon the size of the fish, the smaller ones being more susceptible to heavy infections. He apparently bases his conclusions on the general appearance and host behavior. Hunter (1936) records experiments in which the liver of the common sunfish was infected by strigeid metacercariae resulting from exposure to the cercariae of *Neascus vanclaevei*. Later (1937) the same author noted that it was possible to produce a fatal infection with this parasite. In such cases the liver, spleen, kidney and pericardial cavity were completely riddled by metacercariae of this species. One of the first attempts to study the effect of strigeid metacercariae on the weight of their fish host under experimental conditions, however, was briefly recorded by the Hunters in 1936.

#### PROCEDURE

In order to obtain evidence of the effect of parasites upon the weight of fish a group of No. 4, hatchery-raised fingerlings of the small-mouthed black bass (*Micropterus dolomieu*) were divided into two lots and kept in aquaria in the same laboratory.<sup>2</sup> The temperature of the water ranged 19–22° C during the experiment.

The problem of weighing the fish with a fair degree of accuracy was accomplished by cooling the water with ice cubes, so that the fish were quite immobile. They were then carefully removed, and excess water was blotted off, and the animal was then placed in a dish whose weight had been determined previously, and weighed. Each fish was reweighed twice within a few minutes as a check. The results corresponded so closely that it was not felt necessary to continue this triple weighing.

The fish were weighed at the beginning of the experiment and weekly thereafter. They were marked and all fed the same amount of food per gram weight of fish. The controls were kept in one tank and the experimental fish in another. The tanks were aerated by bubbling air through the water. It is true that each individual fish did not necessarily eat its full share of food each day. It was assumed, however, that this would average out uniformly over a period of several months. Furthermore, the food usually remained in the aquaria for some hours after feeding before its removal. Thus it was available to the smaller fish. Reference to Table 2 indicates the validity of this assumption for only one of the control fish showed a loss of weight, while three remained unchanged and one appreciably gained weight. All of the experimentally infected

<sup>2</sup> These fish were obtained through the courtesy of the New York State Conservation Department.

fish, upon the other hand, showed a loss in weight. All of the fish were about five months old at the start of the experiments.

It should be pointed out that it was intended to use larger numbers of fish, but many had to be eliminated as they died prior to the experiment. The final count showed five control fish and eight experimental fish. The latter were exposed to large numbers of viable cercariae of *C. ambloplitis* from naturally infected snails of the species *Helisoma trivolvis*. Later an infected snail was placed in the aquarium with these experimental fish for twenty-four hours.

#### RESULTS

The No. 4 fingerling, small-mouthed black bass were selected because it was believed that these would be fairly resistant to heavy infections.

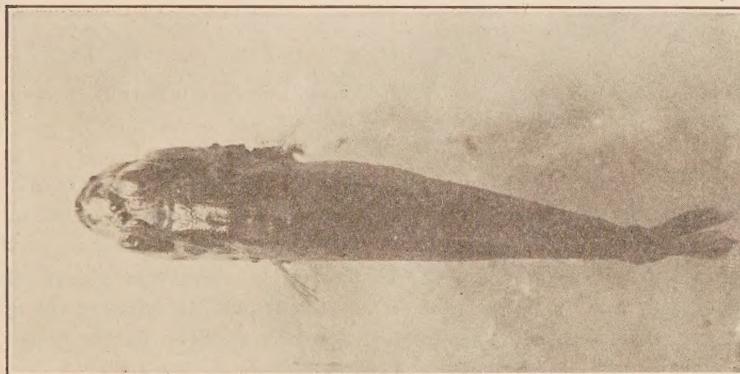


FIG. 1. Experimental fish No. 2 (see Table 1) showing heavy infection.

They were about five months old and none weighed over eleven grams. At the end of three months the experimental fish showed a surprisingly heavy infection from 193 to 708 metacercariae of the black grub of bass, *C. ambloplitis*. The cysts were found in large numbers on the fins and at the base of the tail. Cysts were likewise present in great numbers in the flesh (Fig. 1). The results of the counts are summarized in Table 1.

TABLE 1.—Summary of weights and infection of experimental fish

Fish No.	Weight (gms) at start of expt.	Weight (gms) at end of expt.	Net change (gms)	Number of cysts
1 . . . . .	2.4	1.8	-.6	227
2 . . . . .	5.4	4.9	-.5	not counted*
3 . . . . .	3.3	3.1	-.2	370
4 . . . . .	4.3	3.9	-.4	193
5 . . . . .	9.8	8.1	-.7	568
6 . . . . .	3.3	2.5	-.8	246
7 . . . . .	3.1	2.0	-.1	342
8 . . . . .	5.5	4.7	-.8	708
Average loss..			0.76 ± gm per fish	

\* Saved entire. Equally heavy infection, however.

One experimentally infected fish was saved entire, and the cysts not counted (Fig. 1). The other seven had an average of 392 cysts per fish, truly an appreciably heavy infection for fish of that size and weight. These same experimental fish, although harboring nearly 400 parasites per fish (which must have weighed an appreciable amount themselves) showed a loss in weight of 0.2 to 1.7 gm, an average of  $0.76 \pm$  gm per fish.

The controls changed but little. One lost slightly and the other gained (see Table 2). Although the average gain was  $0.04 \pm$  gm, this change was due to one fish.

TABLE 2.—Summary of weights in grams of control fish<sup>3</sup>

Fish No.	At start of expt.	At end of expt.	Net change
1 . . . . .	4.0	4.0	0.0
2 . . . . .	10.9	10.9	0.0
3 . . . . .	3.0	3.0	0.0
4 . . . . .	2.3	2.2	- 0.1 (loss)
5 . . . . .	3.1	3.4	0.3

Average gain =  $0.04 \pm$  gm per fish. This is, in reality, due to gain in weight in one fish.

#### DISCUSSION

In order to determine whether or not the above differences could be regarded as significant, it was necessary to analyze these data statistically. R. A. Fisher's (1934) method for determining the significance of the difference between the means of the two sets of observations gave such a large value for "t" that it may be concluded that the average changes noted above were significant, and that the differences in weight between the experimental and control fish were valid.<sup>3</sup>

It should also be pointed out that the five control bass were negative at autopsy when the experiment was terminated. A total of 31 fish died prior to the experiment. These were likewise used as controls and were carefully dissected. One of these fish yielded a single encysted metacercaria of the black grub of bass (*C. ambloplitis*). Adding these 31 fish to the five controls, we have one cyst present, or 2.7 per cent, in the 36 control fish. No other parasites were encountered. Surely there can be no doubt but that a significant infection was produced experimentally.

It is suggested that since the experimental fish were active and ate well that the observed loss in weight was an expression of disturbed metabolic processes. This after all may be expected, for besides absorbing nourishment from the host for two months the parasites, which are

<sup>3</sup> Dr. Burton H. Camp, Wesleyan University, kindly analyzed the figures of both tables to determine their statistical significance. He says, "There are various statistical tests which might be applied. Every one of them would show clearly that the difference is significant. For example, Fisher's *t* test would be the test which would be most commonly applied to a case like this. The value of *t* is so large that it is outside Fisher's table, indicating, therefore, a very high degree of significance."

growing rapidly, must in turn liberate their waste metabolic products into the surrounding tissues. Penetration of these cercariae further results in cyst formation taking several weeks, and involving the proliferation of connective tissue and the appearance of melanophores about the cysts.

#### SUMMARY

From the above experiment it may be concluded that a heavy infection of metacercariae of *C. ambloplitis* in young small-mouthed black bass caused a statistically significant loss in weight when compared with the control fish. It is further suggested that this loss is due, in part at least, to a disturbance of the normal metabolism of the host.

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## A PARASITOLOGICAL RECONNAISSANCE IN ALASKA WITH PARTICULAR REFERENCE TO VARYING HARES. II. PARASITOLOGICAL DATA\*

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Incidental to a field survey in Alaska in June and July, 1937, for the possible presence of Rocky Mountain spotted fever in the rabbit tick, a number of birds and animals, particularly varying hares, were taken in various localities. Incidental biological observations on the varying hare are presented in Part I of this paper (Philip, 1939). Acknowledgments of assistance are made in that section.

The parasitological features of the trip are presented here, with emphasis on the varying hare, since that animal was taken in the largest numbers, and since it is of considerable importance in the fur industry, and as food for dogs and even man in the territory.

### PARASITES OF VARYING HARES

From one to many larval *Taenia pisiformis* were found in 29 hares proportionately divided among the several localities (see Table 1). Their situation was most often in the mesenteries near the lower end of the stomach, thrice, a few were also in the liver, and once in the lower pleural cavity only.

TABLE 1.—Number of varying hares with parasites in various localities

Locality	Total hares	<i>Taenia pisiformis</i>	<i>Cittotaenia p. americana</i>	<i>Pasalurus nonannulatus</i>	<i>H. leporis-palustris</i>	<i>H. glacialis lynx</i>
Seward . . .	22	5	5		7	
Anchorage . .	6	3				
Fairbanks . .	70	9	5		40	
Circle Hot Springs . .	5					
Circle . . .	1					1
Rapids . . .	31	7	4			2
Gulkana . . .	16	2	1	1		4
Chitina . . .	14	3	1			14
Totals . . . .	165*	29	16	1	47	22

\* Seven hares were also taken in 3 additional localities bringing the total for the territory to 172.

Mature *Cittotaenia pectinata americana* were encountered in the small intestines of 16 hares taken in 5 localities. In proportion to total

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animals examined, the higher percentage of infection of these tapeworms at Seward and Rapids is noteworthy. Three of the 5 Seward, and one of the 4 Rapids hares had double infections with this and *T. pisiformis*, while another of the Rapids animals was only three-quarters grown; all other *Cittotaenia* hosts were adult hares. In a few instances, *T. pisiformis* also were taken in immature animals.

The small nematode, *Passalurus nonannulatus* was found once only; this animal was taken at Gulkana and the infection was extremely heavy. Although every stomach was examined, no *Obeliscoides cuniculi* were seen; time was not taken to look for *Dirofilaria scapiceps* (Leidy) in the subcutaneous tissues of the tibio-metatarsal joints.

Infestations of the rabbit-tick *Haemaphysalis leporis-palustris* were remarkably localized. At Seward, 7 of the 22 hares carried only one to 3 ticks (mostly adults but an occasional nymph) which were invariably attached among the bristles on either side the nose. These were reluctant to detach even after 36 to 48 hours cooling. Only one other area provided infested hares, namely that adjoining a few miles of the so-called "College Farm-loop road" about 5 to 8 miles west and north of Fairbanks. Collections in other directions from that community and often not far distant from tick-infested areas were negative, although hares themselves appeared no less abundant. Of 46 taken along the "Loop-road," 39 carried various stages of rabbit ticks. It is of interest to note that, whereas 9 of the 14 total ticks taken near Seward, June 12 to 19, were female ticks in various stages of engorgement, among those taken in the "Loop-road" area about a month later were only 6 females (one dead *in situ*), but 245 males as well as 220 nymphs and 314 larvae; in the latter locality 42 males, 15 nymphs and 31 larvae was the maximum collection taken from one hare.

It is known (Philip, 1937) that, in a naturally occurring population in Montana of the Rocky Mountain wood tick, *Dermacentor andersoni*, the males predominate early in the season, the females toward the end, and that while the males tend to remain on infested hosts for some time, the females complete their engorgement and drop off for oviposition. Available information indicates that the normal, adult tick-season for the rabbit tick is extended beyond that of the above species. The Seward data and observations of Mr. Warwick in the Fairbanks area before the arrival of the writer suggest that the preponderance of males (244 to only 5 living females on 39 "Loop-road" hares) at the time of visit was likely due to their persistence on the hosts after the females had largely dropped. The locality should provide an excellent opportunity for future observation on the seasonal distribution and destiny (increasing or decreasing) of a probably temporarily restricted population of this important tick species. In numbers, the present infestations were

far below many encountered on hares in the northern United States where single animals may carry hundreds or even several thousand (Green and Schillinger, 1935).

The distribution of the rabbit flea, *Hoplopsyllus glacialis lynx*, was also peculiar. None was found on any hares from the Kenai to Fairbanks, but east on the Steese highway to Circle, 5 were recovered from one animal at Deadwood near Circle Hot Springs, and 2 more from a hare near Circle. No more were encountered before reaching Rapids, where 2 of 31 hares carried one specimen each. Passing down the Richardson Highway, 4 of 16 hares at Gulkana carried 2 to 6 fleas each, while at Chitina every one of 14 animals was infested with one to 6 fleas.

Two mites were taken from a hare near Fairbanks, and one specimen of the blackfly, *Simulium venustum*, on an animal at Gulkana. Blackflies were not encountered anywhere in the abundance anticipated, but mosquitoes of several species were extremely abundant during the late hours of collecting in several localities and hares were often noticed shaking their heads as though flapping the ears to dislodge such visitors. Since records of mosquito attack on rabbits in nature have not been noticed, but are important in connection with possible disease transmission, it is of interest to record that those insects were quite willing to bite shot hares while still warm during the process of bagging.

Observed pathological conditions included:—two hares taken at Gulkana, one with catarrhal enteritis of the duodenum (diagnosis by Lillie), the other with caseation of the lower right lung and adhesions to the pleural wall, with concomitant purulent appendicitis, and thickening and inflammation of the wall of the appendix; one barren female at Chitina, with a group of dark non-parasitic cysts in connective tissue in the posterior abdominal cavity; and one each at Seward and Fairbanks with severe swelling and necrosis about the joint of one hind leg as though resulting from an old injury. Six animals shot near Fairbanks also showed distinctly enlarged spleens, and in one instance the liver also was enlarged and rather friable; the spleen of one of these measured  $70 \times 14 \times 7$  mm., and tularaemia infected ticks were recovered from this animal as mentioned below. In none of these, however, were necrotic foci visible.

#### LABORATORY STUDIES

Living rabbit ticks from the Seward and Fairbanks areas, although disappointingly few in number, were periodically forwarded to the Rocky Mountain Laboratory of the Public Health Service to be inoculated into healthy guinea pigs for infectivity tests. Partial results reported elsewhere (Philip and Parker, 1938) showed that *Bact. tularensis* was recovered in certain tests for the first time from the territory, while

TABLE 2.—*Host-parasite data (other than of varying hares) from various localities in Alaska, June-July, 1937*

Host	Endoparasite and location	Ectoparasite	Locality	Date
<b>MAMMALIA</b>				
1. Dall sheep <i>Ovis dalli</i> (Nelson)	<i>Taenia hydatigena</i> mesenteries	<i>Oropsylla</i> n. sp.	Rapids	July 19
2. Wolverine, <i>Gulo luscus</i> (Lin.)	<i>Taenia foiecheli</i> Schwartz	<i>Thrasis acomantis</i>	do	July 18
3. Hoary marmot, <i>Marmota caligata</i> <i>caligata</i> (Eisch)	<i>Dianarya</i> sp. (probably <i>composita</i> Darrah), small intestine	<i>Anoplura</i> (undet.)	Seward (fleas) Mills Creek & Rapids (tapes only)	June 15 July 1 and 16
4. Ochraceous marmot, <i>Monax monax ochracea</i> Swarth.	<i>Ascaris tarbagana</i> Schultz, small intestine	<i>Oropsylla aecomys</i> (Baker) <i>Monosyllus vison</i> (Baker)	Fairbanks	July 18
5. Yukon ground squirrel, <i>Citellus plesius osgoodi</i> (Merriam)	<i>Oropsylla</i> n. sp. <i>Anoplura</i> (undet.)	<i>Oropsylla</i> n. sp.	Circle Hot Springs, Rapids	July 10, 15-17
6. Alaska red squirrel, <i>Sciurus hudsonicus</i> <i>petulans</i> Osgood	<i>Orchopeas caedens</i> (Jordan) <i>Monosyllus vison</i> (Baker)	<i>Orchopeas caedens</i> <i>Monosyllus vison</i> (Baker)	Seward and Skilak Lake, Skilak Lake (fleas); Fair- banks	June 9, 12-18, 20 & 25
7. Pika, <i>Ochotona collaris</i> (Nelson)	<i>Dermatoxys</i> sp., intestine	<i>Millettia</i> sp.	Rapids	July 16
8. Meadow mouse, <i>Microtus</i> sp. (probably <i>operarius</i> )		<i>Megabothris querini</i> Roths.	Fairbanks, Rapids	July 13
9. Dawson red-backed mouse, <i>Clethrionomys dawsoni</i> <i>dawsoni</i> (Merriam)		<i>M. querini</i> Roths. <i>Malarctes penicilliger</i> Gr. <i>Ixodes angustus</i> Neum.	Rapids; Mills Creek, and Skilak Lake	July 17 June 15 and 25
<b>AVES</b>				
1. Double-crested cormorant, <i>Phalacrocorax auritus</i> (Lesson), and nest.	<i>Contracaecum</i> sp. (prob. <i>spiculigerum</i> ) gullet	<i>Ceratophyllus niger</i> Fox	Skilak Lake	June 25
2. Herring gull, <i>Larus</i> <i>argentatus</i> <i>smithsonianus</i>		<i>C. niger</i> Fox	do	do
3. Coots, and nest.	Nothing found on bird or in nest		Gulkana	July 21
4. Arctic tern, <i>Sterna</i> <i>paradisea</i> Brun.	Nothing found			
5. Golden eagle, <i>Aquila</i> <i>chrysaetos</i> (Linn.)			Rapids	July 18
6. Ptarmigan nest (occupied), <i>Lagopus</i> <i>leucurus</i> Swains. & Rich.			Mills Creek	June 28

others indicated the possible presence of a low-grade type of Rocky Mountain spotted fever similar to that previously reported by Parker (1935) as associated with this tick species.

If tularaemia has been responsible for any considerable decimation among the hares, it is remarkable that no human cases of disease in Alaska have been recognized, considering the frequency of contact. Agglutination tests on the serums of older Indians in the territory might furnish interesting data in this connection.

#### PARASITES OF OTHER VERTEBRATES

A list of parasites and other hosts taken incidental to the hare studies is presented in Table 2. Included in the list is one apparently new species of fleas. Of interest also is the northward extension of the known ranges of the rodent tick, *Ixodes angustus*, and the flies, *Protophyliphora* sp. whose blood-sucking maggots infest the nests of certain birds. These flies were found near Rapids in the nest of the golden eagle.

#### SUMMARY

1. Of 172 varying hares taken at various places in Alaska, 29 carried larval tapeworms, *Taenia pisiformis*, and 16 were infected with adult *Citotaenia pectinata americana*; 4 were double infections with both. The nematode, *Passalurus nonannulatus*, was recovered once.

2. Ectoparasites included a few rabbit ticks, *Haemaphysalis leporis-palustris* on 7 hares near Seward, and moderate numbers on 40 hares near Fairbanks under very restricted circumstances. Fleas, *Hoplopsyllus glacialis lynx* were chiefly restricted to hares of the lower Richardson Highway.

3. Laboratory studies of the rabbit ticks, limited by paucity of material, revealed the presence of tularaemia in the territory for the first time, and also suggested the possible occurrence of low-grade type of Rocky Mountain spotted fever.

4. Consideration of the limited distribution of these ectoparasites suggests the unlikelihood that arthropod-borne infection could be a factor in the impending decline in population.

5. Parasite records for certain other birds and animals are included.

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# LIFE HISTORY STUDIES OF NORTH AMERICAN FRESH-WATER POLYSTOMES<sup>1</sup>

ALLARD ANTHONY PAUL

## I. INTRODUCTION

### A. Life Histories

Information concerning the life cycles of monogenetic trematodes is very limited. Although observations upon various members have been reported, the literature before 1933 contains complete life histories of only four species. Of these, Zeller (1872, 1876) described *Diplozoon paradoxum* and *Polystoma integerrimum*; Katheriner (1904), *Gyrodactylus elegans*; and Jahn and Kuhn (1932) the fourth, *Epibdella melleni*. The first two species belong in the POLYOPISTHOCOTYLINEA, the third in the MONOPISTHODISCINEA, while the fourth is assigned to the MONOPISTHOCOTYLINEA. *Epibdella melleni* is a marine form, and the others are fresh-water species. All have ciliated larvae except *Gyrodactylus*, which is viviparous. The accounts cited above were reviewed by Alvey (1936).

In a preliminary note, Alvey (1933) described briefly the life history of *Sphyranura oligorchis*, a parasite on the gills of *Necturus maculosus*, and a more complete account is given in his later paper. The eggs are laid and fall to the bottom of the pond, where the larvae develop and emerge. The larvae, which are not ciliated, creep or swim by means of the caudal disc for about twenty hours after emergence, and upon coming in contact with a *Necturus*, attach and migrate over the skin to the gills, where development is completed. Maturity is attained in less than two months.

Gallien (1932a, 1932b, 1933, 1934b) published brief reports and a monograph (1935) which contained a résumé of previous papers and a complete account of his experimental research upon the biology and dimorphic development of *Polystoma integerrimum*. Gallien corrected and amplified the work of Zeller (1872, 1876) on the anatomy, physiology and life cycle of this species. Essentially, the life cycle is as follows: adults, in the urinary bladder of *Rana temporaria*, lay eggs only in the spring of the year. These eggs give rise to larvae which attach upon the

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Thanks are expressed to Professor A. L. Melander for the original specimens of *Hyla versicolor* in which *Polystoma integerrimum nearcticum* was discovered and for his continued interest and support; to Dr. G. K. Noble for laboratory facilities at the American Museum of Natural History during the summer of 1934; and finally to Professor H. W. Stunkard, under whose direction the study was made, for invaluable and inspiring guidance at every step.

internal gills of the tadpoles of *R. temporaria*. The tadpoles are generally in an advanced state of growth, i.e., over three weeks of age. The larvae remain in an arrested state on the gills until metamorphosis of the tadpoles. At this time, they migrate through the alimentary canal to the recently developed urinary bladder. Egg-production begins in the spring of the fourth year. If, however, the larvae fix upon young tadpoles the development is considerably accelerated and modified, and the parasite produces eggs at the end of five weeks, while it is still within the branchial chamber. This branchial generation differs strikingly from the bladder generation. The cirrus is rudimentary and there are no vaginae, vaginal ducts or long coiled uterus; nor is there a genito-intestinal canal. The form emerges and dies at the time of metamorphosis of the tadpoles. The larvae of this form are indistinguishable from those which emerge from the eggs of the bladder form, and they attach to gills of older tadpoles and mature later in the bladder. Thus, at least with the larvae from the bladder form, two cycles are possible: one direct, omitting the mature branchial form; the other indirect, including the mature branchial form. Whether the monogenetic or the digenetic cycle is taken by the larvae of the bladder form depends upon the age of the tadpole at the time of attachment.

Since the larvae from the branchial form are indistinguishable from those of the bladder form, it was logical to assume that under the same conditions at the time of attachment, they might present a second branchial generation instead of remaining in an arrested state of growth until metamorphosis. If this phenomenon occurred, there would be a third type of life cycle besides the two already discussed. However, it does not happen in nature that tadpoles between 8 and 13 days of age are present when larvae of the branchial generation emerge. Gallien delayed the development of frog eggs through reduced temperatures, but the larvae of the branchial form did not give rise to a second gill generation. Gallien states concerning the larvae of the two forms, "Si la morphologie est identique, la physiologie ne l'est pas. . . ."

Gallien noted morphological and physiological differences which exist between the adult form in the bladder and that in the branchial chamber. He discussed the sexual cycle in the former, pointed out its parallelism with that of the frog host and, due to this correlation, stressed the simultaneous oviposition of the host and parasite as an important factor in the perpetuation of the parasite. He postulated a hormone action to regulate the time of sexual maturity; since the parasite is sanguivorous and feeds on the blood of the host, a single substance would induce sexual maturity in both animals simultaneously. There is no comparable cycle in the branchial form, which is distinguished by intense nutrition, acceleration of growth and a marked modification of anatomy and physiology.

Wilde (1936) reported a sixth monogenetic life history, that of the monopisthodiscinean *Dactylogyrus macracanthus*, from the gills of a fresh-water fish. The mode of development of *D. macracanthus*, as reported by Wilde, is essentially like that of *Sphyranura oligorchis*. Eggs are laid; free-swimming ciliated larvae emerge; and if they are successful in coming in contact with a suitable host, migrate to the gills and develop.

In addition to the accounts of complete life cycles in the monogenetic group, there have been other observations. In the MONOPISTHODISCINEA, Kulwieć (1927) described the free-swimming stage of *Dactylogyrus crassus* and the development of *D. anchoratus* after attachment. Siwak (1931) discussed the egg, larva and early stages after attachment of *Ancyrocephalus vistulensis*, a parasite on the gills of *Silurus*.

In the POLYOPISTHOCOTYLINEA, observations of life history have been made by Stunkard, Ozaki, and Gallien. Stunkard (1924a) discussed the development of *P. (Polystomoides) multifalx* after establishment in the oral cavity, and in particular described the development and morphology of the suckers on the caudal disc. Ozaki (1931, 1935a) described *Diplorchis ranae* from the urinary bladder of *Rana rugosa*. In a brief note (1932), he discussed the life history of this species and (1935b) presented a detailed description of the morphology and behavior of the swimming larva. Gallien (1934a) described the egg and larva of *Dactylocotyle luscae*, a parasite on the gills of a marine fish.

#### B. Taxonomy

Diesing (1859) revised the status of the trematodes, reducing them to the rank of a tribe which included three subtribes: ACOTYLEA, COTYLOPHORA, and PLECTANOPHORA. With experimental demonstration of the "direct" development of the many-suckered ectoparasitic trematodes and the "indirect" development of the distomes, van Beneden (1858) advocated the revision of the trematode classification and the erection of two groups: MONOGENEA and DIGENEA. In the MONOGENEA he recognized two families: the TRISTOMIDAE with a single posterior sucker, and the POLYSTOMIDAE with several posterior suckers. In the POLYSTOMIDAE he included the genera *Polystoma*, *Diplozoon*, *Gyrodactylus*, and others.

Fuhrmann (1928) accepted the division of the TREMATODA into MONOGENEA and DIGENEA, and in the former he subdivided Odhner's MONOPISTHOCOTYLEA into two groups, one of which he termed the MONOPISTHODISCINEA. In the suborder POLYOPISTHOCOTYLINEA, Fuhrmann recognized five families: POLYSTOMIDAE, ONCHOCOTYLIDAE, DICLIDOPHORIDAE, OCTOCOTYLIDAE and MICROCOTYLIDAE. In the family POLYSTOMIDAE he included the following genera: *Polystoma*, *Polystomoides*, *Oculotrema* and *Sphyranura*.

Ozaki (1931) erected a new genus *Diplorchis* to contain the new species *D. ranae*, and (1935a) he created another, *Parapolystoma*, to con-

tain the species *Polystoma bulliense* and *P. alluandi*. Both *Diplorchis* and *Parapolystoma* were included in the family POLYSTOMIDAE.

The correlation of structure, life history, habitat, and taxonomy of the TREMATODA presents several interesting features. Because Zeder (1800) confused the anterior and posterior ends of *Polystoma integerrimum*, and mistook the caudal suckers for mouths, the trematode groups have since been inappropriately designated as polystomes, distomes and monostomes. Since the "Mémoire" of van Beneden (1858), the descriptive terms MONOGENEA and DIGENEA have been constantly employed. The former term denotes a cycle of only one generation which is regarded as *direct* development; the latter, the alternation of two or more generations designated as *indirect* development. Nevertheless, among the DIGENEA certain of the ASPIDOGASTRIDAE are "monogenetic" and within the MONOGENEA, *Polystoma integerrimum* may be and usually is "digenetic." In general the monogenetic species are ectoparasitic whereas the digenetic ones are endoparasitic; this correlation is necessitated by the peculiarities of the life cycle. However, the separation of trematodes upon the basis of ectoparasitism and endoparasitism is as loose as the meaning of the terms. It is arbitrary and not always easy to allocate a specimen. For example, some authors consider parasites *on* the gills as ectoparasites, whereas others identify those *within* the branchial chamber as endoparasites. A parasite of the alimentary canal is not *within* the body, in the same sense as one which invades the tissues.

Among the TURBELLARIA certain species have a genito-intestinal canal, whereas it is absent in others. Correspondingly, a genito-intestinal canal is present in certain of the MONOGENEA and absent in others. It is generally agreed that trematodes are descended from turbellarian-like ancestors and consequently, in agreement with Odhner's classification of the MONOGENEA which emphasized the presence or absence of a genito-intestinal canal, it has been considered that the monogenetic suborders constitute two naturally distinct groups which have arisen from two lines of turbellarian ancestry, one with and the other without a genito-intestinal canal. However, in the same genus, and moreover, in the same species, *Polystoma integerrimum*, there is a bladder form with a genito-intestinal canal and a branchial form without one. This situation may invalidate the present concept of origin and relationship.

The apparent discrepancies in correlation of structure, life history, habitat and taxonomy of certain trematodes as revealed by these citations, emphasize the importance of further study upon these subjects and their interrelations.

## II. MATERIALS AND METHODS

Two species of polystomes, different in structure, life cycle and habitat, have been studied. The first, the only form reported from AMPHIBIA

in North America, was briefly described and named, *Polystoma integerrimum nearcticum* n. subsp. (Paul, 1935). It is a parasite of the tree toad<sup>2</sup> and has been found in two species of *Hyla*. It occurs in two forms, one (Fig. 1) of which infects the urinary bladder of adult tree toads; the other (Fig. 2), the gills of larval stages. Specimens were obtained from *Hyla versicolor* in New England and from *H. cinerea* in Florida. More than half of the specimens of *H. versicolor* were infected. The usual number of adult worms in the bladder is two or three. The second species (Fig. 3) has been mentioned only once, in an abstract (Paul, 1936). It does not agree with the description of any known species and the name *Polystomoides oris* is proposed for it. This species infects the oral cavity and has been found rather frequently in *Chrysemys picta* from a pond near Cold Spring, New York. The greatest number found in one turtle was five, three adults and two immature forms. More often, two or three were present, occasionally at different degrees of development. Of 57 turtles, 18 were parasitized; and from them, a total of 40 worms was obtained.

To study the life history of *Polystoma integerrimum nearcticum*, about 350 specimens of *Hyla versicolor* (Le Conte), their eggs and larvae were collected at Weston, Connecticut, during the breeding season in the years 1934, 1935 and 1936. The toads were isolated in beakers which were fitted with cloth covers and contained a half-inch of spring-water to receive the wastes voided. The animals adhered to the wall of the vessels well above the waterline. The method prevented the desiccation of worm eggs released and facilitated their collection at selected intervals. The eggs, which were released abundantly and in masses, were removed to clean pond-water by means of a capillary pipette. This method permitted observations upon the developing larvae and furnished eggs for the experiments. Tadpoles of *H. versicolor*, raised in the laboratory from eggs, were subjected to infection, and observations were made upon the development of the parasites on the gills. Eggs from the gill generation were also abundant and these were treated in the same manner. No serious difficulty was encountered. Tadpoles of *H. versicolor* collected in Connecticut were naturally infected with gill forms of *P. integerrimum nearcticum*, and these worms were compared with specimens produced under experimental conditions. Although one of three specimens of *H. cinerea* from Florida harbored the parasite within its urinary bladder and half of the specimens of *H. versicolor* from Connecticut were parasitized, all other amphibians from the Connecticut area proved negative. Twenty specimens of *H. crucifer*, twenty-four of *Rana clamitans*, thirty of *R. pipiens* and seven of *R. catesbeiana* were dissected in a search for the parasites.

<sup>2</sup> HYLIDAE show no close relationship to the POLYPEDATIDAE; they have evolved from bufonids, not ranids. See Noble (1931), Biology of the Amphibia.

For life history studies of *Polystomoides oris*, specimens of *Chrysemys picta* were collected from the Barrett Pond, near Cold Spring, N. Y., decapitated, and the polystomes removed and identified. Egg-producing worms were transferred to the oral cavity of other turtles which were then isolated. By this method it was possible to concentrate and maintain a larger supply of gravid worms as a source for the eggs. Because egg-production is slow and only one egg is released at a time, it was difficult to secure enough eggs in the same stage of development for experimental infection. Perhaps the difference in rate of egg-production between the gill form of *P. integerrimum nearcticum* and *Polystomoides oris* is due to the difference in food supply, for the vascularity of the gills is much greater than that of the oral mucosa. Another possible explanation may be found in the amount of ovarian tissue, for that in the branchial form of *Polystoma integerrimum nearcticum* is fully three times as great as that of *Polystomoides oris*, and ripe ova are more numerous. The eggs falling from the mouth had acquired a mucus coating during their brief stay within the oral cavity. This substance permitted the attachment and growth of molds which destroyed the eggs. Although a larva occasionally succeeded in attaining a state of growth suitable for emergence, it could not escape since the operculum was closed by the network of mold. Sterile media were provided, but in practice it was found impossible to wash the eggs free of this contaminated coating. Thus, to eliminate the great loss of eggs, they were placed in vessels on a shaker which kept the water constantly agitated and the eggs rolling. By this means, no growth of mold arose, as the egg surface was continuously rubbed smooth. The eggs were examined at selected intervals in order to watch the development of the larvae. Eggs of *Polystoma integerrimum nearcticum*, released through the cloaca from the bladder, or by way of the spiracle from the branchial chamber, were not mucus-coated and therefore, unlike the eggs of *Polystomoides oris*, presented no difficulties in hatching.

To infect turtles (*C. picta*), it was desirable to have a small amount of water and yet keep the animals submerged. Under such confinement the host makes a better target for the swimming larvae. Turtles were placed in small dishes in shallow water, and eggs from which larvae were emerging were introduced. These turtles were decapitated later and examined for attached larvae. The oral and esophageal linings were dissected from the head and transferred immediately to a small paraffined dish containing a thin layer of equal parts of Ringer's solution (frog) and pond-water. Here the tissue was spread out under tension by means of pins, and a search made for the larvae under direct illumination with a low-power binocular dissecting microscope. Even with extreme care, it was almost impossible to locate any of the minute larvae on the oral

or esophageal epithelium. It was discovered, however, that the gradual addition of chloretone caused the larvae to release their oral suckers and raise and twist their bodies at an angle to the surface of the epithelium. Thus they were more readily located, and then removed and studied alive, or in the fixed condition. Later, older stages were obtained.

Observations were made on living and fixed material of both *Polystoma integerrimum nearcticum* and *Polystomoides oris*. Whole mounts, stained with paracarmine (Mayer's) were made of the various stages. The internal structures were studied from frontal, sagittal, and transverse serial sections. They were cut at a thickness of 7 or 10  $\mu$ , stained with Harris' or Delafield's hematoxylin, and counterstained with eosin. Two whole mounts of *Polystoma integerrimum* from Europe were borrowed through the courtesy of Prof. H. W. Stunkard for comparison with *P. integerrimum nearcticum*.

### III. OBSERVATIONS

#### *A. Polystoma integerrimum nearcticum*

A detailed account of the anatomy of the genus *Polystoma* has been given by both Zeller (1872, 1876) and Gallien (1935). In a natural genus no morphological disagreement is to be encountered and except for the enumeration of specific features it is deemed unnecessary to present a detailed description of *P. integerrimum nearcticum*.

##### Bladder form (Fig. 1)

*Specific diagnosis:* Based on measurements of 12 adult specimens. Body muscular, ovoid, 2.5–4.5 mm, av. 3.6 mm long; 0.9–1.5 mm, av. 1.2 mm wide. Caudal disc with six suckers, cordiform, 0.5–0.8 mm long; 0.8–1.7 mm wide. Suckers with wall not divided into zones; diameter 0.15–0.30 mm, av. 0.26 mm. One pair large definitive hooks present, larval hooks usually absent. Definitive hooks falciform, incised and crested. Well marked vaginal swellings shortly behind the level of the pharynx. Oral sucker subterminal; pharynx pyriform, 0.15–0.20 mm in diameter. Esophagus absent. Intestinal ceca extend within caudal disc, are greatly lobulated laterally and communicate medially by anastomoses without specific pattern.

*Male organs:* Testis multilobulate, situated ventrally, extending from vitello-vaginal ducts to caudal disc. Genital pore median, slightly anterior to vaginae. Cirrus spines usually 8, occasionally 9.

*Female organs:* Ovary comma-shaped, usually on right side, size variable, 0.25–0.75 mm long. Vaginae lateral, immediately behind genital pore. Genito-intestinal canal present on ovarian side. Vitellaria extend throughout body. Uterus long and convoluted, containing many eggs. Eggs ovoid, approximately 300 by 150  $\mu$ .

*Host:* *Hyla versicolor* and *H. cinerea*.

*Habitat:* Urinary bladder.

*Locality:* Weston, Connecticut.

*Type specimens:* Am. Mus. Nat. Hist., New York.

The bladder generation of this species differs from that of *P. integerrimum* Frölich, in geographical location, habitat, size, and in the

character of the intestinal anastomoses. In *P. integerrimum* there are, typically, three transverse commissures which form a distinct and characteristic pattern between the intestinal crura. These commissures are absent in *P. integerrimum nearcticum* and the intestinal branches anastomose at random between the crura.

#### Branchial form (Fig. 2)

**Specific diagnosis:** Based on measurements of 10 adult specimens. Body fusiform 1.64–5.0 mm, av. 2.04 mm long; 0.29–0.76 mm, av. 0.45 mm wide. Musculature weak. Caudal disc cordiform, not well set off from body proper; suckers, six in number, pedunculate. Suckers with wall not divided into zones; diameter 0.11–0.15 mm, av. 0.12 mm. Great hooks if present, rudimentary; larval hooks usually displaced when present. Oral sucker subterminal; pharynx spherical 0.09–0.13 mm, av. 0.10 mm in diameter. Gut saccate anterior to vitelline collecting ducts and reticulate caudad.

**Male organs:** Testis spherical, immediately behind junction of vitelline ducts. Cirrus rudimentary, immediately anterior to ootype; spines 8.

**Female organs:** Ovary elongate, medial, 0.29–0.53 mm, av. 0.41 mm long. Vaginae, uterus and genito-intestinal canal absent. Egg ovoid, approximately 300 by 150  $\mu$ .

**Host:** *Hyla versicolor* and *H. cinerea*.

**Habitat:** Gills of larvae.

**Locality:** Weston, Connecticut.

**Cotype specimen:** Am. Mus. Nat. Hist., New York.

This branchial generation of *P. integerrimum nearcticum* differs from that of *P. integerrimum* in the character of the intestine. In *P. integerrimum* the gut is entirely saccate (Gallien, 1935), whereas in *P. integerrimum nearcticum* it is saccate only anteriorly, being reticulate posteriorly.

Since *Polystoma integerrimum nearcticum* from the American toads of the genus *Hyla* differs from *P. integerrimum* of the European frog *Rana temporaria* in the pattern of the gut, and moreover is not found parasitizing any forms of the genus *Rana* which is represented in the same locality, it is concluded that *P. integerrimum nearcticum* is distinct from *P. integerrimum* and entitled to the status of a subspecies rather than a variety.

#### Life Cycle and Development

In the bladder generation of *Polystoma integerrimum*, the reciprocal copulation of two individuals has been clearly described (Zeller, 1876) and confirmed (Gallien, 1935). The latter author has added an account of external self-fertilization. In the branchial generation there are no external vaginae, and the process is described as internal self-fertilization. The writer has not observed these processes in *Polystoma integerrimum nearcticum* but the methods are probably the same.

The eggs of the bladder form of *P. integerrimum nearcticum* are indistinguishable from those of *P. integerrimum*. The latter have been

described by Willemoes-Suhm (1872) and Zeller (1872). Generally, the eggs of *P. integerrimum nearcticum* are ellipsoidal, 300 by 150  $\mu$ , but there is great variation in size and shape. The shell is amber in color with a knob-like process at one end; at the opposite pole, before hatching, an operculum appears. The egg is so filled that the globular yolk-cells are crowded into an orderly pattern of polygons. In their midst lies the ovum, approximately 45  $\mu$  in diameter.

Egg deposition by *Polystoma integerrimum nearcticum* has not been observed. Zeller (1872) thought that the adult of *P. integerrimum* migrated to the cloaca to thrust out its genital orifice and deposit eggs directly in the water. Halkin (1902) disagreed, stating that since the ova are in advanced stages of cleavage, the eggs must be released within the bladder, to be ejected with the urine. Gallien (1935) refuted this hypothesis since he found that, although infrequently ova may have undergone some cleavage before reaching the water, generally they have not. The writer has found that the ova of *P. integerrimum nearcticum* have usually undergone no segmentation when the eggs are examined immediately after deposition in the water. In one case, however, in the uterus of a worm taken from the bladder there were about three dozen eggs containing individuals in all stages of development, from single cells to fully formed larvae. Observations upon isolated toads show that worms do not come into the water to lay eggs and die. It is improbable that they migrate from the bladder for egg deposition and return. To do so would necessitate their return, with the possibility of their losing their way, and no gravid adults were found elsewhere than in the bladder. The eggs are formed consecutively in the specialized region which is anterior to the "shell gland" and pass into the long uterus; but it seems likely that release from the uterus is *en masse*, since many eggs may escape from the toad one day and then none for several days. The number of eggs laid by one worm during the period of production may reach eight hundred. Worms in toads collected in the field usually lay eggs for only a week or two.

The development of the eggs at room temperature is completed in 11 to 13 days. When laid, the eggs are very transparent and the ova easily seen; but on the 2nd and 3rd days the shell has deepened in color, the condition of the yolk material changed and as a result the development of the larvae is obscured. Attempts to fix and section eggs were futile. The shells collapsed in the fixative (Schaudinn's), broke in cutting, and the crumbled yolk failed to hold the larva when sectioned. On the 8th day after laying, the yolk-mass within the egg shows a disturbance at its center, indicating that the larva is becoming active. On the 10th and 11th days the yolk-cells are almost entirely consumed and the larva, bent on itself, can be seen very easily.

The larvae measure approximately 300  $\mu$  in the extended condition and are identical in appearance with the descriptions of *P. integerrimum* as given by Zeller (1872, 1876) and Halkin (1902), except that the rudiments of the great hooks were not observed. The body is flattened dorso-ventrally and bears a circular caudal disc upon whose margin are 16 larval hooklets, 30  $\mu$  in length. At the cephalic end there are two groups of cells which appear glandular in nature. The oral sucker is subterminal and leads into a well-formed pharynx which connects with the developing intestinal crura. On the dorsal surface, cephalad to the pharynx, there are two pairs of eye-spots. Tufts of cilia are visible laterally in the region of the oral sucker, posterior to the pharynx, on the fourth quarter of the body proper and on the posterior portion of the caudal disc. The excretory system is identical in appearance with that described for *P. integerrimum* by Gallien (1935).

On the 12th and 13th days hatching takes place. At this time the limits of the operculum are well defined. The operculum of one egg containing an active larva, observed over a period of several hours, seemed at first to be securely closed. However, the lid opened later and as water entered, the larva became more active. In hatching, the operculum gradually gapes, as though drawn and bent by tension. Previous to emergence the larva contracts, elongates, and performs plunging movements, with the cilia beating continuously. When the lid is sufficiently removed from the circular opening, the larva protrudes its body by degrees until only the caudal disc remains within the shell. Finally, with further raising of the operculum, the released larva darts into the medium. The mechanism of opening of the operculum is as yet unknown. Since it never opens until the larva is mature, the suggestion is made that the cephalic cells of the larva secrete a substance which unseals it. When an egg containing a fully formed and active larva is forced open under pressure, the shell never ruptures at the opercular line. A ragged tear appears at random in the shell and the larva becomes more active in the presence of the entering water.

The larvae, examined after release, have yolk-cells within the alimentary tract. They swim vigorously in an extended condition by means of their cilia, halting momentarily to undergo a number of contractions, and then resuming their progress. Upon striking the edge of the vessel they adhere by means of the oral sucker and execute grappling movements with their caudal armature. After a period of some twenty hours, the specimens slow down considerably and shortly sink to the bottom and disintegrate, beginning with a blistering of the cuticula.

Laboratory-raised tadpoles (*H. versicolor*), 8 to 14 days of age, were placed with larvae of *P. integerrimum nearcticum*. These larvae were obtained from eggs deposited by worms in the bladder of adult toads. At

intervals of three days, tadpoles were removed and dissected; thus all stages of the developing larvae were obtained. This development was entirely comparable to that of the branchial form of *P. integerrimum* as described by Gallien (1935). Branchial forms were mature and produced eggs 22 days after the beginning of the experiment. Parasites in all stages of development were found on the gills. The eggs and larvae of the branchial form are indistinguishable from those of the bladder form and complete their development in the same space of time.

At this stage of the experiment it was impossible to collect toad eggs from which to obtain parasite-free tadpoles, and in spite of the fact that the toads collected previously had been kept in cold storage, all were spent. Eggs of *H. versicolor* collected at an earlier period were also kept in the cold, but the temperature was not low enough to sufficiently retard development. Hence, the experimentally infected tadpoles were allowed to remain with the larvae issuing from the eggs of their parasites.

It was found that some of the eggs are swallowed by the host. Such eggs, recovered from the feces, washed and properly disposed for embryonation, do not develop. Apparently, only those eggs ejected through the spiracle are capable of development. Eggs released directly from the branchial chamber differ so markedly in the organization of their contents from those recovered from the feces that the two kinds can be distinguished easily under low magnification. The contents of the latter are somewhat confluent.

No secondary infection by larvae from eggs of the branchial form could be detected on the gills of these already parasitized tadpoles because it was impossible to distinguish a new generation among worms at such various degrees of development. Larval worms, however, were discovered in the cloaca before metamorphosis and within the bladder soon after its formation. The unmetamorphosed tadpoles had a typically coiled and well-filled gut and the cloaca was inhabited by larvae identical in appearance with those issuing from eggs. Consequently they had not developed in some other location. Eggs of the bladder form liberate larvae which are definitely known to attach to the gills. Hence, it is probable that the larvae found in the cloaca were produced by eggs of the branchial generation. The larvae discovered within the bladder after completion of metamorphosis had attained a size of 0.58 mm and bore the rudiments of the great hooks upon the caudal disc, but no caudal suckers were present.

The fact that larval forms were discovered within the cloaca some time before metamorphosis, and particularly when the tadpoles were still feeding, suggested that entry to the bladder was accomplished by way of the anus. Zeller (1876) and Gallien (1935) stated that the larvae from branchial worms fix upon the gills and later when the gills atrophy, at

metamorphosis, migrate to the bladder by way of the alimentary canal when digestive activity is much reduced. Zeller (1876, p. 268) reported having frequently seen worms, and occasionally as many as a dozen, migrating within the stomach and intestine at metamorphosis. Gallien (1935, p. 58) stated that he had witnessed this migration several times. He cited one experiment in which metamorphosis was induced prematurely with thyroid extract and stated with regard to one of six tadpoles dissected, "je trouve 2 larves dan l'intestin. . . ." The precise location and condition of the worms were not reported. In view of the findings of these authors, numbers of tadpoles in various stages before, during and after metamorphosis were dissected in a search for larvae migrating within the intestine, but none was ever found. The tadpoles were heavily infected and the worms large enough to assure detection, but none was present at any time.

To support the conclusion that migration to the urinary bladder is accomplished via the alimentary canal, Gallien (1935) stressed the point that in nature all worms in the bladder of any one host approach the same state of development. This result would be expected if establishment took place within such a brief and sharply defined period as that which exists when feeding ceases during metamorphosis. However, mature specimens of *Hyla versicolor* collected in the field were found parasitized by both mature and immature worms. In other words, not all worms in the three-year-old toads were mature. This observation is at variance with those of Zeller and Gallien and might be explained on the theory that the toads had been reinfected *anally*, during their annual sojourn at the breeding places. At any rate, the observations of Zeller and Gallien are not confirmed and the possibility of entrance to the bladder by both the intestine and anus must be recognized.

The development in the bladder parallels that of *P. integerrimum* as described by Gallien (1935). An experimentally infected toad, metamorphosed nine days, had four larval worms in its bladder, and of these one had the posterior pair of suckers already formed. Another toad, metamorphosed 17 days, had seven larval worms in its bladder, all with posterior suckers. Toads one year old harbored immature worms (1.5 mm long) with all three pairs of suckers present. None of the pairs of suckers had attained their maximum growth; the posterior pair, the first to arise, were considerably more advanced than the anterior pair, which are the last to arise. Much general growth has taken place by this time, but the reproductive apparatus is in a very rudimentary state.

Inasmuch as the egg-laying of the bladder form is correlated with that of the host, it is probable that maturity is reached in the third year, when the toad becomes mature. In mature toads at the time of laying, all mature polystomes also lay eggs, though for a limited time. This corre-

lation of oviposition by host and parasite was explained by Gallien on the basis of a hormone present in the blood of the host. Since the worms are sanguivorous, the substance operates on the reproductive organs of both. My observations support this suggestion of Gallien.

*B. Polystomoides oris n. sp.*

(Fig. 3)

A detailed account of the general anatomy of species belonging to the genus *Polystomoides* was given by Stunkard (1917). The form described below agrees, except in specific features.

*Specific diagnosis:* Based on measurements of 12 adult specimens. Body fusiform. Length 2.9–4.2 mm, av. 3.6 mm; width 0.8–1.6 mm, av. 1.1 mm. Caudal disc cordiform, 0.72–1.10 mm in length, 0.95–1.30 mm in width; bears six suckers (diameter 0.30–0.36 mm) with wall divided into three zones. Six larval hooks between the anterior two suckers; one at the base of each sucker; four larval hooks, one pair of great hooks and one pair of intermediate hooks, between posterior suckers. Great hooks falciform, bearing incision but no crest. Vaginae open on sides ventrally, a little less than one-third the distance of the body from the anterior end. Oral sucker subterminal; pharynx ellipsoidal 0.50–0.65 mm in diameter; esophagus short. Intestinal ceca, with prominent diverticula anteriorly, extend to caudal disc without anastomoses.

*Male organs:* Testis, ellipsoidal, in middle of body proper, 0.20–0.45 mm by 0.24–0.50 mm. Genital pore directly behind intestinal bifurcation. Cirrus spines 24 to 28, usual number 27.

*Female organs:* Ovary comma-shaped, usually on right, 0.09–0.26 mm. Genito-intestinal canal present on same side as ovary. Uterus absent. Oötype opposite ovary, contains one egg. Vitellaria consist of an aggregation of globular follicles, surrounding the lateral and ventral side of the crura, extend from the pharyngeal level to the end of ceca; behind the testis they coalesce in the median line. Egg ovoid, approximately 0.25 by 0.18 mm.

*Host:* *Chrysemys picta*.

*Habitat:* Oral cavity.

*Locality:* Cold Spring, New York.

*Type specimen:* Am. Mus. Nat. Hist., New York.

This species differs from all previously described members of the genus *Polystomoides* in three features: (1) relative size of pharynx; (2) the form of the intestine; and (3) the number of cirrus spines.

*Life Cycle and Development*

In *Polystomoides oris*, egg-production occurs throughout the year. Copulation was never observed but it was noticed that eggs gathered from a turtle harboring only one mature worm did not produce larvae. However, the introduction of a second mature worm resulted in the production of a considerable number of eggs which underwent normal development and from which swimming larvae emerged.

The collection of eggs from numbers of individuals indicates an average yield of two or three eggs per worm over a period of twenty-four hours.

The eggs measure approximately 250 by 180  $\mu$ . In a small percentage of the eggs there is a miniature knob-like process of shell material on the antopercular end. The operculum is invisible in newly-laid eggs but it becomes conspicuous when the larva is about to emerge. The eggs are a light amber color which deepens as the days pass during embryonation.

At the time of extrusion the ovum is unsegmented and visible within the yolk-mass, which is clear at this early stage. The ovum is approximately 42  $\mu$  in diameter. Cleavage cannot be followed, since the transparent nature of the yolk is so quickly altered. After 11 days of embryonation the larval adhesive disc, with its 16 hooklets and the rudiments of the four definitive hooks, is clearly distinguishable. At 14 days the larvae are well-formed and may be seen silhouetted behind the diminishing yolk-cells. About the 26th day, the larvae (Fig. 4) have consumed nearly all the yolk-cells and become active. Just before hatching, the beating of the cilia can be seen. Emergence occurs usually around the 28th day, although the rate of development is dependent upon the temperature.

The released larvae, when fixed, measure on the average 275  $\mu$  in length and 65  $\mu$  in width. The diameter of the adhesive disc is approximately 84  $\mu$ . Except for the presence of the paired intermediate and great hooks, which measure 35 to 40  $\mu$  in length (Figs. 5, 6), they are indistinguishable from the larvae of *P. integerrimum nearcticum*.

The behavior of the larvae upon release is the same as that of *P. integerrimum nearcticum*.

A number of infection experiments were performed. In each of the first three, twelve larvae were employed. Two days after exposure the turtles were examined and the parasites removed. In experiment I, three worms were recovered from the oral cavity; in experiment II, one worm; and in experiment III, two. The percentage of larvae which succeeded in attaching was exceedingly low, i.e., 15 to 20%. It was not feasible to attempt a repetition of the above experiments using greater numbers of larvae, since it is so difficult to procure them.

The larvae, in this short period of attachment within the oral cavity, lose their cilia and many obtain blood.

An identical experiment was performed, but the larvae allowed to remain within the oral cavity for six months, and two worms were found. They were similar in appearance, with the posterior suckers well formed and the median pair in process of formation (Fig. 7). At this stage of development the larval eyes have disappeared; but all hooks, both larval and definitive, are still functional. The genital primordium is visible as a post-pharyngeal condensation and the cells which are to form the vitello-vaginal ducts are growing laterally. The oral sucker and pharynx

are enlarging and the digestive crura are lengthening. The two posterior suckers, although complete in their architecture, have not reached their maximum size.

Further observations on the development of *P. oris* were made upon immature specimens collected in nature. The rate of growth as observed in individuals reared under experimental conditions indicates that at least a year is required for the attainment of maturity.

The definitive structure of caudal suckers characterizing the genus *Polystomoides*, and their development in *P. multifalx* (Stunkard, 1924), were described by Stunkard in 1917 and 1924 respectively. The writer is unable to add to this account through his observations upon *P. oris* except to state that the units in the cuticular band between the intermediate and distal zones (Stunkard, 1917) of the rudimentary sucker do not increase in number as growth occurs.

During the 4-sucker stage, the genital primordia grow rapidly so that by the advent of the 6-sucker condition the definitive structures are all outlined in a rudimentary state. The ovary, oötype, vaginae, testis and cirrus are undergoing cellular differentiation and are readily observed. The spines of the cirrus have not attained their full dimensions. The post-pharyngeal diverticula of the gut are now distinctly manifest. By the time the last-formed pair of suckers have become functional, the caudal musculature is strongly developed and the entire caudal armature is operative (Fig. 8).

With further growth and differentiation, maturity is reached and the reproductive structures become functional.

The accompanying table shows the growth of various structures through measurements made in different stages of development.

TABLE 1.—*Measurements of structures in microns*

Stage	Length of hooks			Length of cirrus spines	External diameter of suckers on caudal disc	External diameter of pharynx	External diameter of oral sucker	Length of worm (mm)
	Larval	Intermediate	Great					
Larva ..	30	35	35	..	..	22	52	0.3
2-sucker.	30	55	85	..	73	75	115	0.6
4-sucker.	30	65	100		105	85	120	0.8
6-sucker.	30	65	115	46	175	190	270	1.5
Adult ..	30	65	120	58	315	555	570	3.5

#### IV. DISCUSSION

The type of *Polystoma* is *P. integerrimum* Frölich (1791) from the European frog, *Rana temporaria*. Rudolphi (1819) described a similar species from the oral cavity of the turtle, *Testudo orbicularis*, which he referred to the genus *Polystoma* and which he named *P. ocellatum*. Subsequently, additional species have been referred to the genus *Polystoma*. Stunkard (1917) noted the morphological differences between

the polystomes of frogs and turtles, and especially differences in form of suckers and reproductive organs. He stated, "The wide variation in structure of the members of the genus *Polystoma* cannot be adequately explained through migration, or through differences in the age of the parasite, type of host, or location in the host. In the genus so far as is known, the long uterus containing many eggs is confined to species infecting the urinary bladder of amphibian hosts of the Old World. However in respect to other characters, e.g., the shape of the caudal disc and absence of great hooks, these amphibian forms of the Eastern hemisphere disagree with each other and agree with forms parasitic in the urinary bladder and oral cavity of North American turtles. The turtle parasites have a very similar structure, whether parasitic in the urinary bladder or the pharyngeal cavity. Furthermore, if the observations of Zeller are correct and the individuals of *P. integerrimum* becoming mature on the gills of tadpoles lack external vaginae and have a spherical testis and a single egg in the uterus, one is entirely at a loss to explain the variation existing in the genus." Because of uncertainty regarding the true identity of the branchial generation of *P. integerrimum*, the lack of constancy in the occurrence of a genito-intestinal canal and external vaginae, and variation in the length of the uterus among the known representatives of the genus *Polystoma*, Stunkard (1917) made no attempt to subdivide it.

Ward (1917) proposed the subgenus *Polystomoides* for those species of *Polystoma* which are characterized by a short "uterus" containing one egg, and *P. coronatum* Leidy (1888) was designated as type of the subgenus. Stunkard (1924b) raised the group to generic rank. Fuhrmann (1928) accepted the generic status of *Polystomoides*, and while the taxonomic validity of the genus seems established, the criterion adopted by Ward in erecting the subgenus is not an adequate one, nor is it of fundamental significance. Evidence against the correctness of this criterion is afforded by the fact that the branchial form of *Polystoma integerrimum* and of *P. integerrimum nearcticum* has a short "uterus" while the bladder form has a long uterus. As a matter of fact, it appears that in the literature, the term *uterus* has been incorrectly applied to a structure of much more fundamental importance,—the *oötype*. This organ appears in the same location in both the bladder and gill generation of *Polystoma integerrimum*, *P. integerrimum nearcticum* and also in *Diplorchis ranae* and species of the genus *Polystomoides*. In these various forms this organ performs the same function,—formation of the egg. Apart from the question of homologies of the female ducts in trematodes and cestodes, it would seem that the designation *oötype* is correct on the basis of both morphology and function. Gallien (1935) stated that a uterus is lacking in the branchial generation of *Polystoma*.

*integerrimum*. The writer's observations upon the branchial form of *P. integerrimum nearcticum* confirm Gallien's findings here. Moreover, the writer has observed that the uterus is lacking also in *Polystomoides*. Further, it appears that the uterus is a relatively less important structure, whose function is storage and emission of eggs. In specimens of the genus *Polystomoides* and in the gill generation of *P. integerrimum nearcticum*, the structure designated by the writer as the *oötype* performs the function of the uterus as well, ejecting eggs directly. It is proposed that the true nature of this so-called "uterus" be acknowledged by the use of the more descriptive term *oötype*.

On the basis of added knowledge, it appears that valid criteria for a characterization of the genus *Polystomoides* consist of the three features here noted (reproductive organs cannot be used). *Polystomoides* has: (1) a reptilian host; (2) only one generation; (3) skeletalized suckers (Fig. 3), whereas *Polystoma* has: (1) an amphibian host; (2) often two generations; (3) askeletalized suckers (Fig. 1).

The larvae of the genera *Polystoma* and *Polystomoides* are indistinguishable both in the living and fixed conditions, except for variation in the number and form of hooks.

#### V. SUMMARY

A review of the literature concerning the life cycles and taxonomy of the MONOGENEA is presented. *Polystoma integerrimum nearcticum* and *Polystomoides oris*, n. sp., are described and an account of their development is given.

The life history of *Polystoma integerrimum nearcticum* is compared with that of *P. integerrimum* Frölich as recorded by Zeller (1872, 1876) and Gallien (1935). The possibility that the bladder generation enters by way of the anus is pointed out.

The features which characterize the genus *Polystomoides* are discussed and it is shown that a true uterus is absent in this group.

Of the two forms discussed in this paper, the first normally exists in two generations, one parasitizing the gills of certain anuran larvae and the other, the urinary bladder of adults; and the second species occurs in only one generation, which infects the oral cavity of turtles. The life cycles of both are therefore eminently adapted to their hosts since AMPHIBIA have a gill-bearing period in their development and turtles lack such a stage.

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## EXPLANATION OF PLATES

## ABBREVIATIONS

c. r.	cirrus rudiment
c. s.	caudal sucker
c. t.	ciliary tuft
e. d.	excretory duct
e. v.	excretory vesicle
g.	vacuity
g. c.	genito-intestinal canal
g. h.	great hook
g. p.	genital pore
g. r.	genital rudiment
i.	intestine
i. h.	intermediate hooks
l. d.	larval disc
l. h.	larval hook
m. g.	Mehlis' gland
o.	ovary
od.	oviduct
oe.	esophagus
oo.	oötype
op.	operculum
o. s.	oral sucker
ph.	pharynx
sp.	sperm
te.	testis
ut.	uterus
v. d.	vas deferens
vg.	vagina
vg. d.	vaginal duct
vl.	vitelline duct
vt.	vitellaria
v. v.	vitello-vaginal duct
y. c.	yolk cell

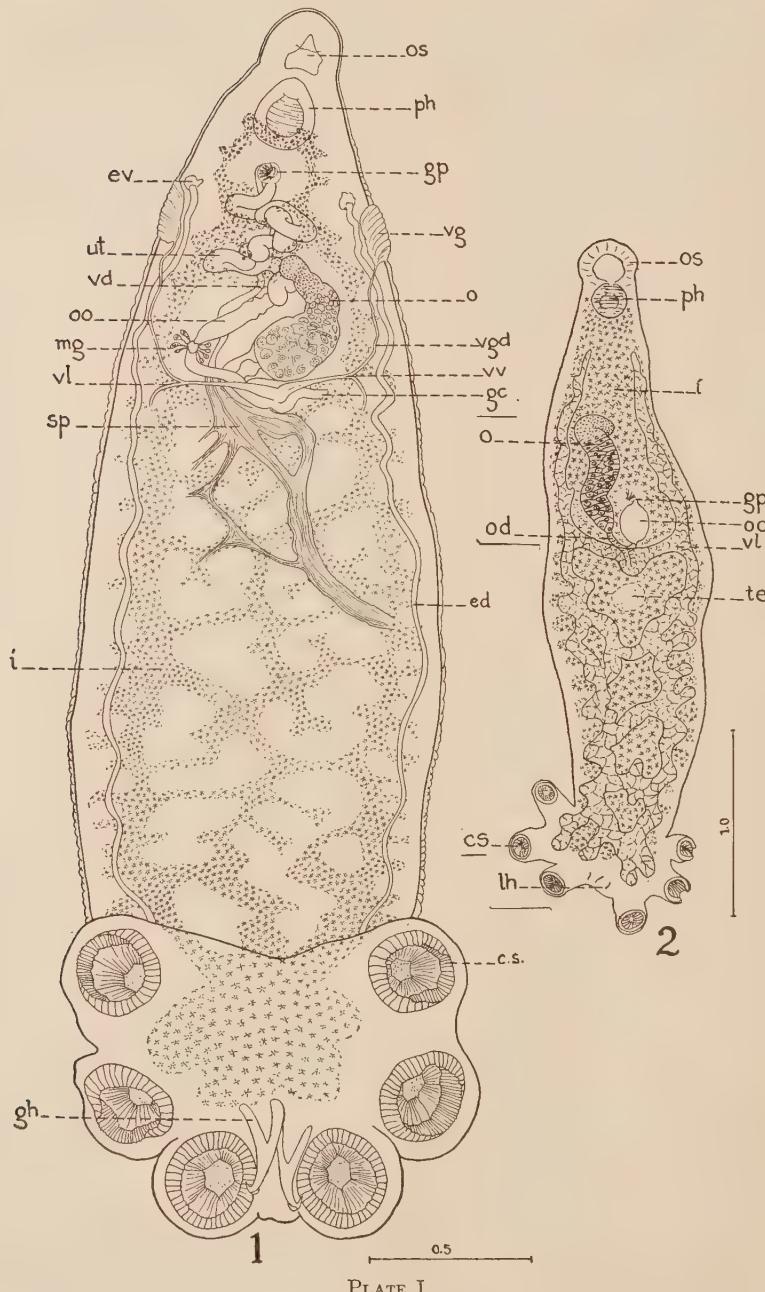
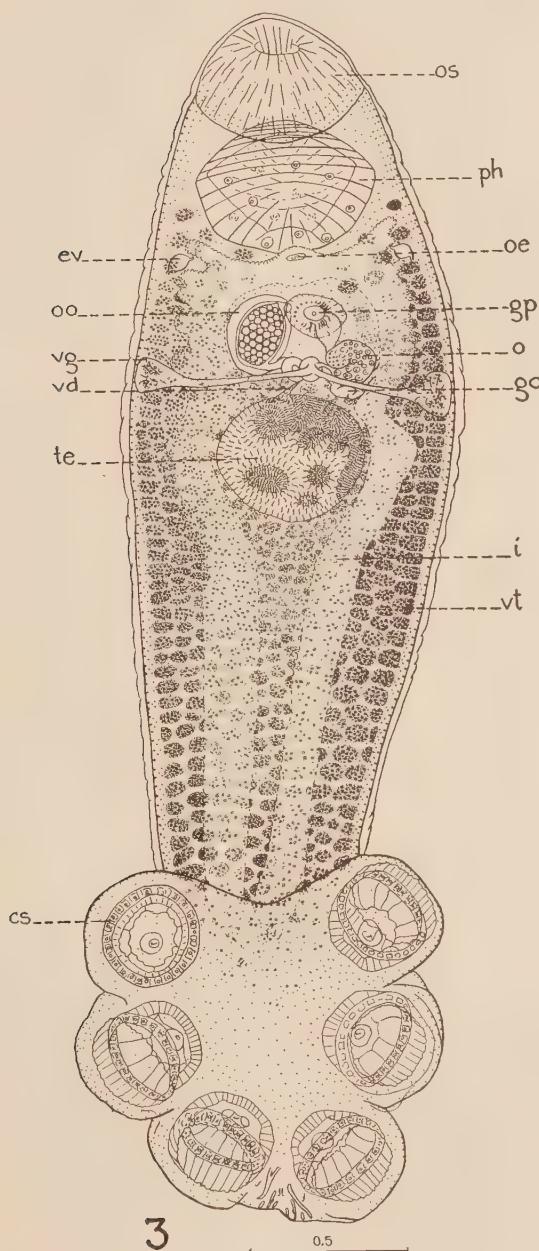


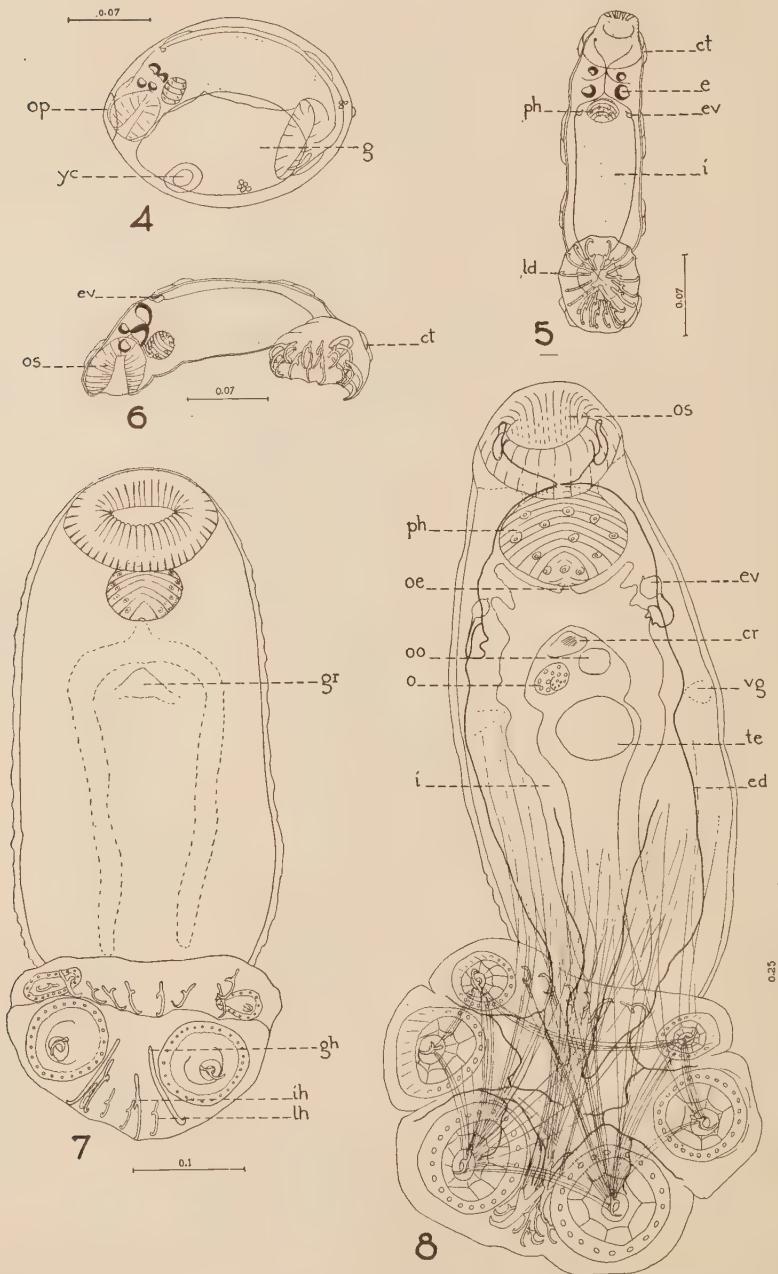
FIG. 1. Type specimen, *Polystoma integerrimum nearcticum* (bladder generation), ventral view, vitellaria and testes omitted.

FIG. 2. Cotype specimen, *Polystoma integerrimum nearcticum* (gill generation), ventral view.



## PLATE II

FIG. 3. Type specimen, *Polystomoides oris*, ventral view.



## PLATE III

FIG. 4. Larva of *Polystomoides oris*, just before hatching.  
 FIG. 5. Larva of *Polystomoides oris*, ventral view.  
 FIG. 6. Larva of *Polystomoides oris*, lateral view.  
 FIG. 7. Early 4-sucker stage of *Polystomoides oris*, ventral view.  
 FIG. 8. Early 6-sucker stage of *Polystomoides oris*, ventral view.

The value of each scale of magnification is indicated in millimeters.

## LOCALIZATION OF *GIARDIA MURIS* IN RATS

ROBERT HEGNER AND LYDIA ESKRIDGE\*

One of the interesting features of the relations between protozoan parasites and their hosts is the localization of the parasite in some particular organ, tissue or type of cell. Apparently very few species of intestinal protozoa are capable of living in the small intestine of certain species of hosts in which many species reside in the large intestine. Thus in man, *Giardia lamblia* is the only species of protozoan that inhabits the lumen of the small intestine, although 5 species of amoebae, 5 species of flagellates and one species of ciliate make their home in the colon. A similar situation exists in rats where *Giardia muris* and *Hexamita muris* are the only common protozoan inhabitants of the small intestine although trichomonads occasionally occur in this division of the digestive tract.

In many textbooks and reference books the duodenum is mentioned as the habitat of *Giardia lamblia*. Thus Wenyon (1926) states that this species "lives in the upper parts of the small intestine." Thomson and Robertson (1929) say that the "duodenum is probably the main site of infection." Manson-Bahr (1936) writes that the "usual habitat of the parasite is in the upper part of the small intestine, but it may also heavily infest the duodenum," and Craig and Faust (1937) state that the trophozoites are "most numerous in the duodenum." It seems quite clear from these and other similar statements that the region of the human small intestine which might be considered the primary site of infection of *Giardia lamblia* has not really been demonstrated with certainty.

Giardias have also been found in the small intestine of many lower animals, in most of which they have been reported from the duodenum. For example, Boeck (1917) states that "the duodenum seems to be the natural habitat" of *Giardia microti* in the meadow mouse, *Microtus c. californicus*. Later (Boeck, 1919) he noted that the jejunum was the principal site of infection. Deschiens (1926) reports *G. cati* from the upper part of the small intestine including the duodenum. Chu (1930) found a heavy infection of *G. hegneri* in the duodenum of a civet cat. Lavier (1935) describes *G. agilis* in large numbers only in the first two centimeters of the small intestine just posterior to the pylorus in frogs of the species *Hyla arborea*.

Faust (1931) examined immediately after death 11 dogs that had been infected per rectum with *G. canis*. He recorded trophozoites from

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the rectum of 8, from the colon of 5, from the cecum of 9, from the appendix of 7, from the posterior part of the ileum of 7, from the anterior part of the ileum of 3, from the jejunum of 1, and from the duodenum of 2. Faust also reports the primary site of infection in dogs harboring a natural infection (number of dogs not given) to be in the cecum and appendix. The distal 10 cm of the ileum was occasionally infected but never the higher levels of the small intestine. Dr. Faust's findings seem rather remarkable to us since we have examined in our laboratory more than 100 dogs infected with giardias and in practically every one the flagellates were most abundant in the anterior portion of the small intestine.

The literature contains many references to the presence of giardias in the bile ducts and gall bladder. Many of these reports are based on the appearance of the flagellates in bile obtained by duodenal tubage. The fallacy of these reports is evident when we consider the fact that *Giardia lamblia* may be an inhabitant of the duodenum. For example, Paulson and Andrews (1930) obtained giardias in the first series of duodenal extractions in each of seven cases in which these flagellates had previously been noted in the feces. Other reports are based on the discovery of the flagellates at the time of surgical operation but the possibility exists that the organisms may not have been inhabitants of the gall bladder but may have been present because of the conditions accompanying the operation.

In a group of 20 rats examined in 1923, *Giardia muris* was reported from the duodenum, jejunum and ileum and the duodenum was considered to be the optimum habitat (Hegner, 1923). Since then hundreds of rats have been examined in this laboratory and while we often find giardias in the duodenum the greatest number seem to be located farther down. This impression is confirmed by the studies of Kofoid, McNeil and Bonestell (1935) who state that in the Norway rat *Giardia* occurs most plentifully in the jejunum at a pH range of 6.45-6.52. Glukhovtzev (1935) states that "the anatomical localization of *G. muris* is preeminently in the upper parts of the small intestine, and of *G. intestinalis*—the lower parts." He seems to have had no difficulty in infecting rats with *G. lamblia* (= *G. intestinalis*) from man. Armaghan (1937) found *G. muris* in laboratory rats to be most abundant in the section of the small intestine that is from 45 to 75 cm posterior to the pylorus, and Hegner and Eskridge (1937) note that the first 18 to 20 cm of the small intestine is usually free from infection. Morenas (1938) finds the jejunum to be the optimum habitat for *G. muris*, *G. simoni* and *G. lamblia*. Giardias were present in the duodenum in 11 of 96 sewer rats and in the jejunum of 29.

The data presented in this paper are the outcome of an attempt to determine more precisely the optimum habitat of *G. muris* in rats. Two

groups of 20 rats each from our colony were used. In the first 20 rats, examinations of material within the lumen and scrapings from the walls gave the following results: from the pylorus to the opening of the biliary duct, all negative; from the opening of the biliary duct to a point 15 cm farther back, all negative; from this point to the cecum, all positive.

The data obtained from the second lot of 20 rats are presented in table 1. The greatest number of trophozoites occurred between 65 and 90 cm posterior to the pylorus, the average being 78 cm. Since the total length of the small intestine in these rats ranged from 102 to 119 cm with an average length of 109.9 cm, the optimum habitat proved to be usually about three-fourths of the length of the intestine posterior to the pylorus; this is in the posterior region of the jejunum. No giardias were observed anterior to the 50 cm level and in only a few rats were specimens noted anterior to the 60 cm level.

Cysts of *Giardia muris* were observed in two rats at the 75 cm level, but were more often found at 90 and 100 cm and in the cecum.

Since trophozoites are probably carried down toward the cecum in the contents of the lumen without encysting, the ileum, no doubt, often contains specimens that are simply passing through. It is, therefore, impossible to determine how frequently this region is actually infected and how many of the flagellates present there may be considered real residents. The region where the first large concentration of giardias posterior to the pylorus occurs seems to us, therefore, to be the true optimum habitat of this species. These concentrations are indicated in table 1 under the heading optimum habitat.

In a recent paper (Hegner and Eskridge, 1937) we reported the absence of giardias from the small intestine anterior to and 15 cm posterior to the entrance of the biliary duct in 18 giardia-infected rats that were fed either on a normal diet or on a diet containing oleic acid; whereas in 9 rats fed on a diet containing bile salts, the giardias extended forward throughout the duodenum. This suggested that a positive reaction had occurred between the bile and the giardias and that entrance into the bile ducts might take place. Accordingly we examined the bile ducts of every giardia-infected rat that we killed, 123 in number, but failed to find any of the flagellates in them. Deschiens (1930) who claims to have found giardias in the biliary tract of man, failed to recover them from the gall bladder or biliary ducts of mice even when the mice were carrying an intense infection. Similarly, Morenas (1938) could not find giardias in the gall bladder of 6 mice with heavy infections and in 6 mice that did not show intestinal infection.

It seems worth while in this place to mention that fact that *Hexamita muris* was present throughout the entire small intestine in most of the 20 rats listed in Table 1.

TABLE I.—Localization of *Giardia muris* in the small intestine of 20 laboratory rats. (- and + refer to trophozoites. C indicates the presence of cysts.)

No. of rat	Length of intestine in cm	Distance in cm posterior to the pylorus										Optimum habitat	Cecum	
		45	50	55	60	65	70	75	80	85	90	95	100	
1	120	-	-	-	-	-	-	-	-	-	-	-	-	C
2	112	-	-	-	-	-	+	+	++	+++	++++	+++++	+	C
3	106	-	-	-	-	-	+	+	++	+++	++++	+++++	+	C
4	108	-	-	-	-	-	+	+	++	+++	++++	+++++	+	C
5	113	-	-	-	-	-	+	+	++	+++	++++	+++++	+	C
6	110	-	-	-	-	-	+	+	++	+++	++++	+++++	+	C
7	113	-	-	-	-	-	-	-	-	-	-	-	-	C
8	105	-	-	-	-	-	+	+	++	+++	++++	+++++	-	C
9	103	-	-	-	-	-	+	+	++	+++	++++	+++++	-	C
10	105	-	-	-	-	-	+	+	++	+++	++++	+++++	-	C
11	102	-	-	-	-	-	+	+	++	+++	++++	+++++	+	C
12	105	-	-	-	-	-	+	+	++	+++	++++	+++++	-	C
13	110	-	-	-	-	-	+	+	++	+++	++++	+++++	-	C
14	106	-	-	-	-	-	-	-	-	-	-	-	-	C
15	112	-	-	-	-	-	-	-	-	-	-	-	-	C
16	117	-	-	-	-	-	-	-	-	-	-	-	-	C
17	119	-	-	-	-	-	-	-	-	-	-	-	-	C
18	115	-	-	-	-	-	-	-	-	-	-	-	-	C
19	105	-	-	-	-	-	+	+	++	+++	++++	+++++	+	C
20	113	-	-	-	-	-	+	+	++	+++	++++	+++++	+	C
Av. 109.9		2+	3+	10+	8+	15+	5+	15+	6+	17+	3+	7+	Av. 78	11+

## SUMMARY

The distribution of *Giardia muris* was determined in 40 giardia-infected laboratory rats that were fed on a normal diet. In 20 of these, no giardias were found in the small intestine between the stomach and a point 15 cm posterior to the biliary duct; all were positive beyond this point. In the other twenty the small intestine ranged from 102 to 119 cm in length, with an average length of 109.9 cm. No giardias were observed anterior to the 50 cm level. The optimum habitat ranged from 60 to 90 cm posterior to the pylorus, with an average of 78 cm. Cysts were observed at the 75 cm level and posteriorly, occurring most often at 90 or 100 cm and in the cecum. The bile ducts of 123 rats were examined but no giardias were found, indicating that migration into the bile ducts does not usually take place.

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THE RATE OF EGG PRODUCTION OF *STRONGYLUS*  
*EQUINUS* AND *STRONGYLUS VULGARIS* AS  
MEASURED BY EGG COUNTS AND  
QUALITATIVE LARVAL  
CULTURES

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In 1923 Stoll demonstrated a correlation between the number of eggs per gram of feces and the number of female hookworms harbored by the human host, thereby greatly enhancing the value of egg counting methods in determining the severity of infection in hookworm areas, and to a lesser extent, in infected individuals. Since that time a great deal of work has been conducted to examine the correlations between egg counts and parasites harbored by man and his domestic animals. Very little information is available regarding the egg production of the horse strongyles, but it is generally conceded that the rate of oviposition of the more than forty species of strongyles parasitising the intestinal tract of EQUIDAE varies considerably. Consequently, egg counting methods alone reveal no accurate index of egg production of the worms and other technics must be looked for to supply this information. This article is intended to show the egg production of *Strongylus vulgaris* and *Strongylus equinus* by egg counts in connection with qualitative larval culture methods.

METHODS

Stoll's original dilution technic (1923 a) was used exclusively in making the egg counts here reported. In a previous report Britton (1937) suggested the value of this technic in studies with horse feces and further work (unpublished) has confirmed the earlier results and indicates the superiority of this method over the flotation technics.

The culture method employed has been a modification of a technic devised by Leiper (1937) in which the larvae may be recovered practically free from debris. After making an egg count of the sample, the fecal balls are broken up and placed loosely in a Petri dish of half-inch depth, leaving an area in the center of the dish free from feces to supply sufficient oxygen and guard against excessive heat production. The culture is incubated for seven days at room temperature and may be left on the laboratory desk if guarded from the direct rays of the sun. At the end of the incubation period, a little 5 per cent formalin is added to the cover on which the larvae may be found in small clumps. The formalin

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serves as a suspension medium for the larvae and also renders them motionless, making the identification easier. A few drops of this suspension are placed on a slide and examined under the low power of the microscope.

The first 100 larvae encountered are identified and then the number of eggs of each species in the total egg count may be estimated by multiplying the percentage of larvae of each species by the total strongyle egg count (EPG). The egg production of each species under consideration was then determined by dividing this number (total EPG  $\times$  percentage of larvae of each species) by the number of adult females of each species recovered at autopsy. The descriptions of the infective horse strongyle larvae given by Lucke (1938), Poluszynski (1930) and Gordon (1933) were followed in the identification of the larvae. Until further studies on the identification of the larvae have been conducted, it is necessary to group all species except *Strongylus vulgaris*, *Strongylus equinus*, *Strongylus edentatus* and *Trichostrongylus axei* into a single group which will be referred to in this paper as the small strongyle group. EPG will refer to the *total* strongyle egg count.

#### RESULTS

In order to determine the accuracy of the results of the qualitative larval culture method, duplicate cultures were made of daily fecal samples from two horses for a period of five days and two or more slides were counted from each formalinized suspension. The results of these preliminary experiments indicated a relative numerical constancy of development in the cultures of the larvae of each species of strongyle. The results of differential larval counts from the slides of both cultures of a single fecal sample never differed by more than 5 per cent for any species and in most cases was much less.

Egg counts and differential larval counts have been correlated with numbers of adult female *Strongylus vulgaris*, *Strongylus equinus*, and *Strongylus edentatus* recovered from 15 horses coming to autopsy. The approximate rate of oviposition of each species has been found to be 1.8 EPG per adult female *Strongylus vulgaris*, 1.8 EPG per adult female *Strongylus equinus* and 2.1 EPG per adult female *Strongylus edentatus* (one case). The figures for *Strongylus vulgaris* and *Strongylus equinus* showed variations ranging from 0.5–3.3 EPG and 0.9–2.9 EPG respectively.

#### DISCUSSION

Under stabilized conditions of worm-host relationships, a knowledge of the rate of egg production might conceivably be used to determine the relative numbers and types of strongyles present in the colon of a horse. By multiplying the percentage of larvae of each species by the total stron-



FIG. 1. Photomicrograph of formalin killed infective horse strongyle larvae, taken under low power. Left to right: *Strongylus vulgaris*, *Strongylus equinus* and a small strongyle.

gyle EPG and dividing the result by the average figure for egg production of each species, the theoretical infection in the group of 15 horses under consideration was deduced. The number of males present was determined by dividing the number of theoretical females by the average sex ratio for each species (Britton, 1937). In this way the theoretical infection was found to account for 90 per cent of the actual *Strongylus equinus* infection, and, excluding one horse in which the majority of the *Strongylus vulgaris* were immature, 100 per cent of the *Strongylus vulgaris* infection.

The results were less satisfactory when applied to the individual horses due to the rather wide variations in rate of egg production which were obtained. The results of experiments recently completed suggest that normal intra-monthly fluctuations unrelated to environmental conditions occur in the egg outputs of the horse strongyles and it is possible that some of the variation is due to the examination of fecal samples during a period of decreased egg production.

It is possible that the method of egg counts and qualitative larval culture may be of value in conducting anthelmintic studies with horses

since there would be no need of killing the experimental animals except when final confirmation is needed. According to Mönnig (1931), who has indicated the potential value of similar technics to studies with sheep parasites, useless drugs show up clearly while useful ones can be detected and retested carefully. Other potential uses for an egg count-larval culture method and a knowledge of the rate of egg production of the various species of strongyles include the possibility of selecting horses for parasitological studies and obtaining a more complete diagnosis of equine strongylosis.

#### SUMMARY

1. The egg laying capacities of adult female *Strongylus vulgaris*, *Strongylus equinus* and *Strongylus edentatus* have been determined by means of an egg count-larval culture method and post-mortem examinations for worms.
2. The average rate of egg production of *Strongylus vulgaris*, *Strongylus equinus* and *Strongylus edentatus* respectively were 1.8, 1.8 and 2.1 EPG per adult female.

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FURTHER NOTES ON THE OVERWINTERING OF THE  
EGGS OF *ANOPHELES WALKERI* THEOBALD  
WITH A DESCRIPTION OF THE EGGS<sup>1</sup>

HERBERT S. HURLBUT

Observations on the occurrence of two types of eggs of *Anopheles walkeri* and data indicating that one of them is an overwintering egg have been presented recently by Matheson and Hurlbut (1937). The writer records here additional data obtained during the fall, winter and spring of 1936-37.

MATERIALS AND METHODS

The overwintering eggs were obtained September 5-23 from four wild specimens collected at Ithaca, New York. The mosquitoes were confined individually, for oviposition, in small jars in the laboratory. The eggs of each batch were removed from the jar within 24 hours and placed out-of-doors on the roof of a low north wing, and thus were protected from direct sunlight while exposed to out-of-door temperature. They were left there until spring. Both small enameled cups and Syracuse watch glasses were used as containers, each one being covered with a glass cover to retard evaporation. They were all placed under a small glass aquarium as protection against wind, snow and rain. The eggs were placed on water in the containers and confined within waxed paper rings which floated on the surface.

The temperature data given are air temperatures which have been secured from the records of the U. S. Weather Bureau Station at Ithaca, which is about 100 yards distant.

EXPERIMENTAL OBSERVATIONS

*From oviposition to hatching.* The individuals from which the eggs were obtained oviposited six times, collectively, yielding a total of 568 eggs September 5-23. The average of the mean daily temperatures for the seven days after each oviposition was respectively, 71.7°, 71.7°, 71.7°, 70.9°, 69.7° and 51.1° F. This embraces a period (September 5-30) with a maximum temperature of 89° F and a minimum of 35° F. It should be noted here that eggs of the summer type hatch after about three days when the temperature averages 70° F. A few of the overwintering eggs were dissected three weeks after they had been deposited, and were found to contain living larvae which had apparently completed their embryonic development.

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<sup>1</sup>Contribution from the Department of Entomology, Cornell University. The writer is indebted to Professor Robert Matheson for criticisms and suggestions in the preparation of this paper.

The highest temperature reached over the entire period was 89° F, the lowest -5° F. As changes in the weather occurred, the eggs were, of course, exposed to alternate thawing and freezing. A record of the daily maximum and minimum temperatures for the whole period is given in figure 1.

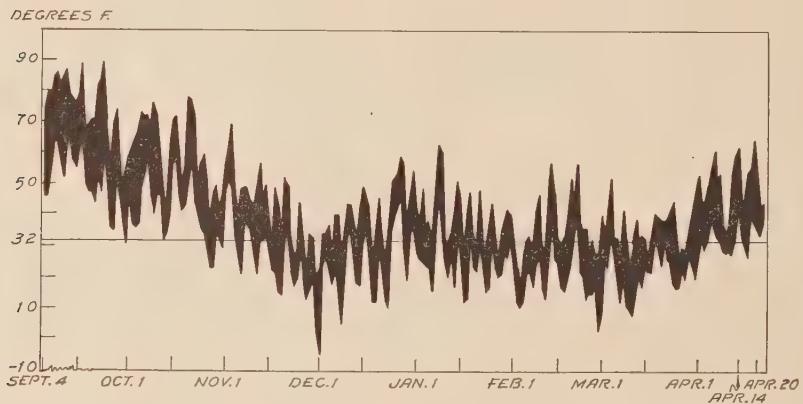


FIGURE 1. Maximum and minimum daily air temperatures at Ithaca, New York, for the experimental period, September 4, 1936 to April 22, 1937.

*Hatching.* Four of the eggs hatched in September a few days after they were deposited. The others remained dormant.<sup>2</sup> In the spring hatching began on April 14 and continued until May 10, when observations were discontinued. Two hundred and one hatched or approximately 35 per cent.

Thirty-five per cent of these hatched during April 19, 20 and 21. This was by far the most pronounced peak that occurred during the hatching period. The mean daily temperatures for these three days were 52°, 46° and 38° F respectively, the maximum 65° F and the minimum 34° F. The average of the mean daily temperatures for the portion of the hatching period preceding was 44° F, with a maximum of 63° F, and a minimum of 27° F. Approximately 87 per cent of the hatching took place April 14-28, during which time the average of the mean daily temperatures was 45.5° F. The average for the preceding 15 days was 39.5° F with a maximum of 61° F and a minimum of 20° F.

<sup>2</sup> In September 1937, 106 eggs of the winter type were obtained from a female collected at Reelfoot Lake, Tennessee. Eleven of these were preserved in formalin. Seventy-two of those that remained hatched within 4 or 5 days in the laboratory at Wilson Dam, Alabama. Fourteen unhatched eggs were recovered from this batch. These remained dormant and were taken to Ithaca, New York, in September. They were placed out-of-doors on October 1 and left there until spring. They began to hatch on March 21. Eleven of the 14 hatched within the next 3 days.

These data were obtained, in part, while the writer was in the service of the Tennessee Valley Authority.

## FIELD OBSERVATIONS

Soon after hatching was observed in April, an attempt was made to obtain larvae in the field. One newly hatched individual was collected on April 17, from a pond where breeding had been observed the preceding summer. At the time of collection, the water at the surface was 48° F. This larva was reared to the third instar, when commonly accepted characters left no doubt as to its identity.

## DESCRIPTION OF THE EGGS

*Summer egg.* The summer egg suggests a boat in its general shape (Plate I, Figs. 1 and 2). Longitudinally, the profile is appreciably concave above, the two ends higher than the mid portion; below, the middle third is more or less straight or slightly concave and the terminal thirds curve strongly upward. Transversely, the egg is flattened above and strongly convex below. Both ends come to a rounded point, but the anterior third is always a little larger. The floats usually occupy a little less than one-half the total length and are medially located a little above the long axis. They possess the regular corrugations common among anopheline eggs.

The delicate external membrane or exochorion is reticulated on the lower surface and on the sides, but on the upper surface it is thin and without reticulations, except in the region between the floats; here it is of the same nature as the sides and lower surface. There is thus a well marked area between the floats, which, however, is usually divided into two parts each paralleling the float of one side but not extending across the median dorsal portion of the egg. The inner border of each part in such cases is marked by an irregular beady line. This line terminates at the ends of the floats but is here continuous with another feature of the exochorion, the so-called *frill* which is a delicate flap 10 to 17 microns wide extending to the tip of the egg. The frill thus borders the upper side of the egg except in the middle, in the region of the floats.

As already stated the exochorion of the upper surface differs in appearance from that of the rest of the egg. It is covered with granular elevations punctured with minute stellate pits; the remaining surface, on the other hand, lacks this granular appearance and possesses rounded rather than angular pits. The reticulations consist of irregular polygons defined by pitted ridges.

Two other features: the micropyle, and the minute circular structures located at the extreme ends of the egg are of the usual form encountered in our species. They have been described in detail by Herms and Frost (1932) for California anophelines.

One hundred eggs from four individuals were measured as to length and width including the floats, and length of floats; and the float corru-

gations counted. The results are summarized in table 1 together with similar data for the overwintering eggs.

TABLE 1.—Summary of measurements in microns and counts of float corrugations of summer and overwintering eggs of *Anopheles walkeri*

	Summer egg (100 eggs, 4 individuals)*			Winter egg (100 eggs, 5 individuals)		
	Mean	Standard deviation	Range	Mean	Standard deviation	Range
Length . . . . .	622	22	577-666	764	51	681-866
Width . . . . .	188	10	163-207	265	11	244-289
Length of floats	292	9	266-325	489	57	385-622
Number of float corrugations .	22.4	1.5	19-27	31.4	1.5	25-36

*Winter egg.* The overwintering eggs differ strikingly from the summer type in the upper surface pattern, general shape, and increased size. They are more nearly circular in cross section because the upper surface is more strongly curved. The longitudinal profile tends to be almost straight, above, or slightly convex (Plate I, Fig. 4); below, it does not differ greatly from that of the summer egg but is somewhat less convex. The floats are relatively longer, occupying a little less than the middle two-thirds of the total length of the egg. They are situated at the level of the long axis rather than above it. The exochorion of the upper surface is reticulated and of the same nature as the sides and lower surface, resembling in surface texture the reticulated exochorion of the summer egg. However, at each end above, there is a small terminal area (Plate I, Fig. 3) which still retains the type of exochorion present on the general upper surface of the summer egg. The frill does not reach the floats but is confined at each end to the border of these terminal areas. The difference in size between the two types of eggs can best be appreciated by examining table 1, and the figures which are drawn to the same scale.

It seems likely that eggs of an intermediate type may occur not uncommonly during a transition period at the season when the change from summer to winter eggs takes place. One example of this kind has already been described and figured by Matheson and Hurlbut (1937).

#### SUMMARY

1. The two types of eggs, summer and winter, of *Anopheles walkeri* are described in detail.
2. Detailed data are presented on the overwintering of *A. walkeri* in the winter egg stage under experimental conditions.
3. The hibernating egg contains a fully developed embryo.
4. Hatching begins about the middle of April when the mean daily temperatures are averaging about 45° F.
5. Evidence is presented that hatching of the eggs of *A. walkeri*

occurs in the field at about the same time as observed under experimental conditions.

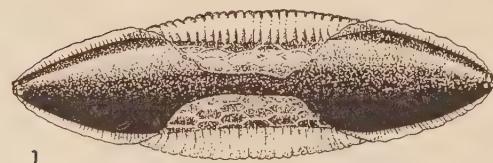
6. It is believed that *A. walkeri* hibernates at Ithaca, New York, in the egg stage. No evidence that it hibernates in any other stage has been found.

7. Winter eggs of *A. walkeri* from Reelfoot Lake, Tennessee, are able to survive the winter at Ithaca, New York.

8. Winter eggs of *A. walkeri* from Reelfoot Lake, Tennessee, exhibited a marked instability in that the greater part of those obtained hatched within a few days after oviposition.

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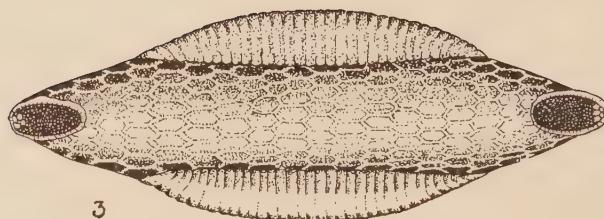
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1



2



3



4

0.3 mm.

## PLATE I

The Eggs of *Anopheles walkeri*

1. Upper surface of summer egg.
2. Lateral surface of summer egg.
3. Upper surface of winter egg.
4. Lateral surface of winter egg.

## TAPEWORM STUDIES. VII. VARIATION IN PASTURE INFESTATION WITH *M. EXPANSA*

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Sheep contract *Moniezia expansa* by foraging where the infective stage is present (Daubney, 1932). Moreover, as sheep graze closely there is a certain inevitability in their encountering whatever parasites are present in a pasture. Taking advantage of this fact, it has been shown by analyzing data from fecal examinations that the infective stage of *M. expansa* has no demonstrable tendency to migrate from infested areas (Stoll, 1935a), and that it persists over long periods when sheep are not present (Stoll, 1935b). While fecal examinations permit the observer to recognize the presence or absence of reproductively mature tapeworms, they give only a small indication of the amount of infestation with which the hosts come in contact. This information can be obtained, however, by another method. If after appropriate exposure in an infested field animals are sacrificed before they become immunized, and examination is made of the intestinal tract, the actual numbers of both mature and immature cestodes which the sheep have contracted may be readily found. There is no multiplication of the parasite in the definitive host. The number of *M. expansa* secured, therefore, when related to the period of exposure, permits determination of the average daily rate of infection and thereby an insight into the changing state of pasture infestation.

Such a study was carried out in our field I-II without interruption for 21 months, covering two complete grazing seasons and the intervening winter. The final picture furnished was of a definite rise and fall in the rate at which sheep contract *M. expansa*, during the time of year they graze, depending on the recency and continuance of renewed pasture contamination by actively infected animals.

Besides adding to our understanding of *Moniezia*, this cestode study has interest in an old epidemiological problem,—that of the amount of infection acquired under natural conditions by susceptible hosts, exposed to the presence of varying concentrations of new infection.

### MATERIALS AND METHODS

Field I-II, the permanent sheep pasture at this laboratory, is mostly a low-lying tract unsuitable for cultivation. It is partly swampy and traversed by a brook. Since 1923 field I, and since 1929 field II, have been infested with *Moniezia expansa*. The flock has had, in general, the

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range of both fields since 1931. This double field, for which a decade of continuous *M. expansa* history through 1932 has been detailed (Stoll, 1935a and b), constituted our test area.

Due to the desirability of reseeding field I, the flock, during the course of our observations, was confined in field II from early September, 1933, to early April, 1934. Shortly after the sheep were re-admitted to field I, that area was enlarged by fencing a contiguous four-acre tract, bringing the total grazing area to  $11\frac{1}{3}$  acres. The new field was higher ground and had not previously been used by sheep. The relationship of these shifts to concentration of animals on an acreage basis is later referred to.

While the sheep had the opportunity of using the pasture in the winter, grazing then was only spasmodic, and ineffective from the standpoint of the present study. Hay was furnished the flock from November to April, the amount being gradually increased as pasturage became inadequate, and gradually decreased in the spring with the growth of grass.

In contrast to the established infestation of *Moniezia* in field I-II, has been the absence of this parasite in animals held indoors, and in several enclosures near the laboratory. *Moniezia* has also been continually absent from our large field VII (Stoll, 1936b) which had been established for cattle and sheep in 1931. In beginning the field VII flock, bottle-raised lambs had been placed there in 1931, increase in 1932 and 1933 being by lambs normally reared on their ewes. In 1933 there were 19 lambs, 7 yearlings and 10 two-year olds, whose availability for introduction to *Moniezia*-infested pasture permitted beginning the present study. Of these, a 7-weeks-old lamb in May and an 8-weeks-old lamb in June were killed as unexposed controls, both being negative for tapeworms post mortem, in agreement with expectation. The remaining 34 animals from field VII constituted the bulk of those introduced into field I during the spring and summer of the first season. Of the 17 additional sheep and lambs placed in field I-II in 1933, and the 30 in 1934, all but 5 were bottle-reared.

Special point is made of the considerable degree of inbreeding in the sheep. Most of our animals had been inbred since 1922, with a few outside ewes added from three different farms in 1929-31. Of the 83 sheep and lambs, over half were sired by ram A, another quarter by his father ram B, the remainder by closely related males. On the female side 4 ewes, their daughters, or granddaughters bore nearly half the animals, while 7 additional ewes or their daughters parented half the remainder. All except 13 of the animals had from 1 to 4 full brothers or sisters likewise under observation; 21 sets of twins and one of triplets were represented. Some of these relationships may be followed in figure 1. While it is doubtful if there is significant gain in expressing these

relationships by an inbreeding coefficient, the degree of homogeneity of the host material seems of consequence in the results.

After placing animals in the test area, some were allowed to remain with the flock as controls, their tapeworm diagnosis to be determined by regular fecal examinations. Others were brought back to the laboratory for sacrifice after varying intervals following pasture exposure. Before slaughter animals were fasted one day, except in 8 instances when the exposed hosts were brought directly from pasture to the autopsy room (noted by absence of stippling in figure 1).

The cestodes were recovered at autopsy by removing the intestine and first opening it carefully to remove large specimens. It was then washed over nested brass screens of 20, 40, and 60 meshes to the inch. The screenings, free of coloring matter and fine debris, were rinsed into large glass culture dishes and examined against a black background for the collection of smaller worms. Final checking was facilitated by adding formalin to the dishes. As we are dealing in this study with the number of parasites contracted, the count given is of scoleces or heads only. Strobilate sizes of *M. expansa* after various periods of exposure of susceptible animals have been elsewhere summarized (Stoll, 1937c).

There are two tapeworm species represented in our infested pasture, but all observations considered in this study deal only with *M. expansa*, as judged by small scoleces, discrete interproglottid glands, and "triangular" eggs. The ripe proglottids appear freely on the fecal pellets, but the presence of segments from the second species, probably *M. benedeni*, is a very rare occurrence. The latter infections are readily distinguished microscopically by their cuboidal eggs, and the strobilae by the presence of linear glands and large scoleces. This differentiation of species appears in some of the fecal examination records of figure 1.

#### RESULTS

Field I-II (also referred to as the pasture, or test area) was known to be infested with *M. expansa* in 1932, and was further contaminated by actively infected (patent) animals with an added supply of tapeworm eggs in June, and September to December, 1932 (Stoll, 1935b). On March 9, 1933, two yearlings were placed in the pasture, and from then on the test area was under observation without interruption until November 23, 1934. The data obtained by determination of the tapeworm status post mortem, or by exposed controls which were allowed time to develop mature tapeworms so that fecal examinations would indicate the presence or absence of infection (in the case of some animals by both methods), are presented primarily in a series of text figures.

Figure 1 shows the consecutive record of animals introduced to the pasture. Besides giving autopsy and fecal examination results, portions



of the bars are shaded to mark the period when individual sheep contracted tapeworms. Parallel shading indicates such determination when based on autopsy findings, diagonal shading when based on fecal examinations. (Cf. Stoll, 1935b, for method of making this calculation.)

The first point made clear in figure 1 is that the pasture was demonstrably infested from March to November, 1933, not provably so December, 1933, to March, 1934, and again demonstrably infested from April to November, 1934. This is shown by positive autopsy results (barring the 6 cases mentioned in next paragraph) in 36 of 42 animals so examined for this purpose, as well as in 38 of 41 animals becoming positive by fecal examination. Eight animals furnished evidence by both methods. Four of the animals which failed to reveal tapeworms at autopsy had been exposed in the winter period, November 23, 1933, to March 31, 1934. One of these, present in the pasture from December 7, 1933, until March 9, 1934, had two fecal examinations each in January, February and March which were likewise negative.

It will be noted in figure 1 that during April to June, 1933, six animals of different ages (reading up on the chart, Nos. 535, 538, 445, 512, 515 and 437) were negative when examined at autopsy within one week of first being exposed in the pasture, although preliminary and parallel controls showed the area positive. The detailed circumstances concerning these, and the additional fact that the 9th day following introduction to pasture is the earliest we have been able to demonstrate

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FIG. 1. A synoptic presentation of *Moniezia* data concerning the susceptible sheep and lambs exposed in field I-II during 1933-34. Each animal is represented by an oblong, which is begun with the exposure date, left unstippled to indicate presence in infested pasture, stippled to show removal from it but still under observation. Following the animal numbers, B stands for a lamb of less than  $\frac{1}{2}$  year age and bottle-raised on cow's milk, S for a suckling lamb (in the 1934 series \* attached to Nos. 599, 600, 601 and 602 indicates they were born on pasture), I a yearling, II a 2-year old. The sex symbol for male castrates (wethers) is omitted, and a short dash used instead. Months are divided into tenths. Triangles represent fecal examinations positive for *M. expansa*, circles negative (squares for *M. benedeni*). With animals no longer in pasture, triangles are left open to call attention to the fact that such infection was not contaminative in the pasture.

The time intervals during which the tapeworm was contracted have been calculated for animals demonstrated positive by fecal examination, and are shown to the nearest 3-day periods by diagonal shading of the oblongs. These bands of shading are shorter and the determinations more precise the more frequent the examinations in relation to effective exposure. Horizontal shading is used for the periods animals were exposed when positive tapeworm status was determined by autopsy only.

Special attention is called to the close kinship of test animals, the striking degree with which controls by fecal examination checked the autopsy determinations, the marked lag in contraction of infection by pasture-born lambs, and, beginning in June, 1933, the continuous contamination of the pasture with *M. expansa* eggs from animals with reproductively mature tapeworms. Data on 81 animals, with 650 fecal examinations and 50 autopsies, are represented.

*Moniezia* in the lumen of the intestine, have already been reported (Stoll, 1937b). They are shown in figure 1 to illustrate their relation to other introduced animals, and the necessity of taking into account this fact of a week's delay in the appearance of tapeworms in the intestine, as judged by routine autopsies.

The data (omitting the 6 mentioned in the preceding paragraph) are replotted in figure 2, to show graphically the numbers of tapeworms found in the autopsied animals. Occasionally more than one susceptible animal was under exposure at the same time. Such duplicates are represented by broken lines. Without reference to the length of time animals were in pasture, the largest number of scoleces found in one host in 1933 was 28; in 1934, 95.

#### *Tapeworms per Day*

In the case of a susceptible animal, it is obvious that differences in the number of tapeworms contracted will depend on the total length of time the animal has been permitted to graze in the infested area, other conditions being equal. Accordingly the data shown in figure 2 have been made comparable by calculating the number of tapeworms secured daily by each host for the period of its effective exposure (upper graph, Fig. 3). By effective exposure is meant the period of grazing up to 9 days before examination post mortem. Thus the first two sheep, Nos. 487 and 494, whose records are shown for 1933, were placed in pasture March 9 and withdrawn April 7. Each was in the area 29 days. The former animal was killed 4 days after withdrawal from the pasture, the latter 25 days; effective exposure for No. 487 was accordingly 24 days, for No. 494 its full pasture period of 29 days. Failure to detect worms in the intestine until the 9th day whether due to biological or to technical reasons, necessarily imposes a correction for the effective exposure period.

The question naturally arises as to whether the graph (Fig. 3) showing the number of tapeworms contracted on a daily basis reflects the actual state of the pasture infestation. There are three types of collateral evidence bearing on this point. One has to do with the age of the sheep hosts when they were exposed in pasture. Another with the degree of crowding in the area,—competition for food and thus for infective tape-worm stages. A third has to do with the results obtained on duplicate test animals. These are next considered.

1. The middle graph of figure 3 illustrates the age of the animals whose autopsy examinations furnished the data. It is clear at once that age of host is not the responsible factor influencing the rate of acquisition of the tapeworms. Thus one of the peak rates in the spring of 1933 was by a 2-year-old ewe, a second similar peak by an 11-weeks-old lamb;

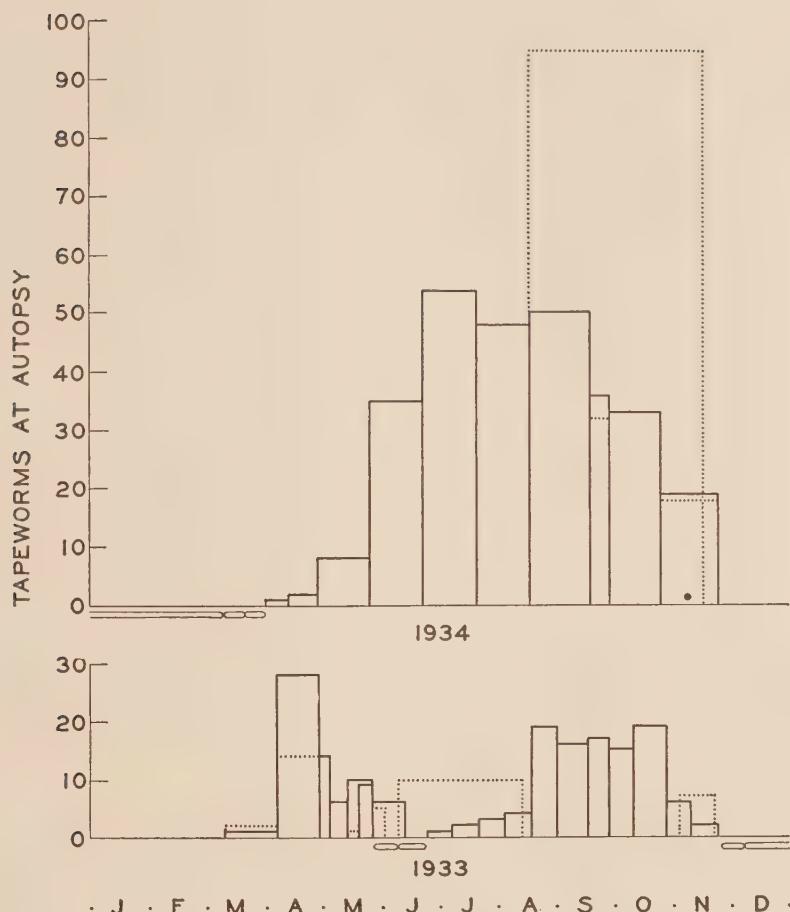


FIG. 2. Results of *M. expansa* worm counts on the 43 sheep and lambs brought to autopsy nine or more days after first being placed in the infested field, shown in relation to their full exposure periods in pasture. (Individual hosts may be identified by cross-reference to figure 1.) Dotted lines represent supplementary test animals; horizontal oblongs below the base lines, three negatives each in 1933 and 1934. The small black circle shown for November, 1934, indicates the contraction of one *M. expansa* as a result of one day's pasturing by yearling 582 (Fig. 1). The bimodal character of the curve for 1933 as compared to 1934 is more accurately defined in the upper graph, figure 3.

an old ewe in the June to August period acquired cestodes at about the same rate as 4 lambs 11–21 weeks of age; the secondary high rate in the late summer and fall of 1933 is not essentially dissimilar for 4 lambs and a ewe 1½ years old; the drop late in 1933 and subsequent failure to show any infection was by sheep of the latter age or greater; the high point in the 1934 infection rate was by animals of approximately similar age to those exposed both before and after this time.

2. A second factor possibly influencing the rate of acquiring tapeworms by animals exposed in the pasture was the concentration of sheep in the grazing area, shown in the lower graph of figure 3. This takes into account not only the changes in the total acreage to which the flock had access, but changes in number of sheep due to the presence of stock animals, which, having had *M. expansa* in some preceding season, were now immune. If concentration of animals were primarily responsible in this study for changes in the rate of acquiring tapeworms, theoretically lower concentrations per acre (i.e., more grazing space per animal and thus less severe cropping of forage) would tend to decrease the number of parasites secured, and vice versa. In such case, the number of tapeworms contracted daily by the sheep would be in no necessary relation to the actual supply available in the pasture, but be due to the differential sampling of such an infestation due to crowding of the host animals. Actually, as the graph reveals, the daily tapeworm rate did not rise with increased sheep concentration in the area. Indeed, the rise and fall and renewal of rise in rate in the spring of 1933 was during a period of decreasing number of sheep per acre; the secondary rise in the tapeworm rate in 1933 began before rather than after increased sheep concentration; this secondary level was maintained and then fell off during a sustained high sheep concentration in the area; while during the changes in rate from May to November, 1934, sheep concentration was approximately unchanged. Competition in grazing, within the limits of this study, was not correlated, therefore, with changes in the daily rate with which tapeworms were acquired by the sheep.

Semple, Vinall, Enlow, and Woodward (1934) in their "Pasture Handbook" state: "An acre of good arable land used exclusively for sheep will ordinarily support from 3 to 5 ewes with their lambs until the latter are marketed." It will be noted from figure 3 that during the actual grazing seasons of 1933 and 1934 the sheep concentration in field I-II was for the most part in this range, and thus represented approximately standard grazing conditions.

3. When more than one animal was exposed in pasture at the same time, in general there was great similarity in their rate of acquiring worms for their effective exposure periods. These duplicate test animals are represented by dotted lines in the upper graph of figure 3. Thus in March, 1933, each of two sheep secured infection at slightly less than 0.1 tapeworm daily; in mid-April each of two at 1.3 and 1.4 tapeworms daily; in late September, 1934, each of two sheep at 2.9 and 3.3 tapeworms daily; in November, 1934, each of two sheep at 0.6 and 0.7 tapeworms daily, during a period when another animal (No. 582) exposed one day only secured just one tapeworm (Stoll, 1936a). The only exception appears to have occurred near the end of May, 1933, when of three 11-

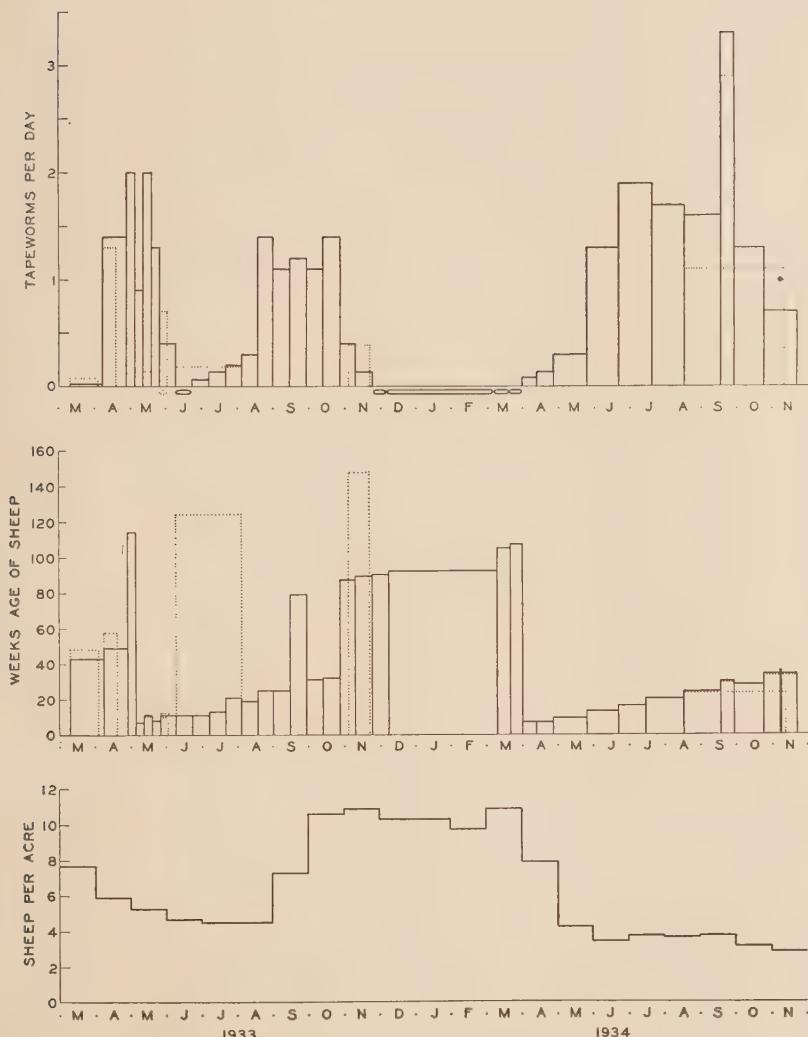


FIG. 3. The tapeworms per day data (upper) shown in relation to age of individual sheep hosts (middle) and concentration of sheep per acre of pasture (lower). In the upper figure polygons of daily numbers of tapeworms contracted represent the tapeworm totals shown in figures 1 and 2, divided by the number of days the animals were on pasture, up to nine days before autopsy (see text). Dotted lines refer to the animals similarly represented in figure 2.

The recession in the curve of tapeworms acquired daily in the early summer of 1933, as compared to 1934, is clear. Changes in the tapeworm curve are manifestly in no constant relation to the age of the sheep, nor to their concentration on pasture.

weeks-old suckling lambs placed in the test area with their ewes for effective exposures of 6 to 14 days, two showed respectively 0.7 and 0.4 tapeworms acquired daily, and the third was negative. This exception occurred during a time when the curve of acquiring the parasites was rapidly falling. Near the end of the 1933 season a 2-year-old ewe which had earlier been positive and later lost her infection indoors, was again exposed and showed an acquisition rate November 3-21 of 0.4 tapeworms per day. It is of interest, in this rare case of non-immunization at first exposure, that the ewe's second exposure period overlapped that of two animals one of which likewise secured 0.4, the other 0.1 tapeworms daily.

In none of the above cases were animals in pasture longer than about a month. As the period of susceptibility to the tapeworms is increased when the host is suffering from heavy worm burdens due to nematodes, two cases of more extended tapeworm acquisitions were observable in animals succumbing to nematode infections. In 1933 the 2-year-old ewe 425 placed in the area June 8 died August 12, and showed 10 *M. expansa*. Her daily rate of acquiring tapeworms for an effective exposure of 56 days at 0.2 daily was in the range of the four 11-21 weeks-old lambs which spanned the identical period and showed in sequence none, 0.1, 0.1, and 0.2 tapeworms daily. Similarly in 1934, a 24-weeks-old ewe introduced to pasture August 16 was moribund when withdrawn November 15. At autopsy 5 days later 95 *M. expansa* were recovered, with a daily rate of 1.1 tapeworms, for an effective exposure of 87 days. This was during a period when other animals exposed secured, in sequence, 1.6, 2.9 (duplicate: 3.3), 1.3, 0.7 (duplicate: 0.6) and 1.0 tapeworms daily, with a weighted average of 1.4 demonstrable for the 3 months involved. There is thus striking similarity in the general number of tapeworms secured by animals exposed at the same or approximately the same time.

As tested therefore by age of host failing to account for the daily tapeworm rate, of sheep concentration within the limits of this study failing to influence such rate, and from the similarity of the number of tapeworms secured by duplicate test animals, the daily tapeworm rates are acceptable as an actual reflection of the state of the pasture infestation.

#### *Relation to New Tapeworm Contamination*

With this evident reliability of the upper graph of figure 3 as a representation of the state of the pasture infestation, its relation to deposition in the area of new *Moniezia* contamination is of essential interest. During the winter 1932-33 there were no sheep in the pasture with active (patent) *M. expansa* infections. Not until early June were infected animals again contaminating the pasture with tapeworm eggs. From then until November, 1934, such contamination was continuous (Fig. 1).

It was our practice during routine fecal examinations of pasture animals to egg count all infections. From these data a curve has been constructed (middle graph, Fig. 4) showing the rate of deposition in the area of newly contaminating eggs. At its peak in July and early August, 1933, over 35 million *M. expansa* eggs were being deposited each 3 days (an interval chosen for convenience in plotting). In August and September, 1934, concentrations in excess of this rate were reached. For most of each grazing season, once animals with mature infections were present, the rate for each 3 days was 10 to 20 million (or more) eggs, except at rare intervals.

It is obvious that the amount of new contamination has a definite relation to the daily tapeworm curve (reproduced as lower graph of Fig. 4). Following its initial rise in April, 1933, and its high point in May, there was a decrease in the curve so that it reached very low levels. While it may not have reached a true zero point, the successive low worm counts in June and July indicated a state of near exhaustion of the pasture infestation, following normal overwintering (Stoll, 1935b). As the middle graph of figure 4 illustrates, new tapeworm eggs began to reach the pasture in early June although such increment was not large until July. There was no marked increase, however, in new infection observable in animals until several weeks afterward. Once re-established the infested state stayed at an approximate level through October. The subsequent falling off in rate which obtained through the winter was related to feeding of hay and consequent absence of grazing, as earlier noted. In 1934, by contrast, once the season's normal pasture infestation was demonstrable (its level approximated that of the preceding year), no early summer recession toward zero infestation was apparent. During late September, a marked increase over the normal rate occurred. Each of these results is anticipated in the curve showing the millions of new eggs reaching pasture: there was no interruption during the 1933-34 winter and spring of contamination reaching the area (a condition opposite to that of the preceding season); there was a temporary recession in new eggs in July, 1934, that may have been reflected in a drop of the tapeworm curve in August; and there was a heavier deposition of tapeworm eggs in August and September, 1934, followed by a marked rise of tapeworms secured by the exposed animals in late September.

The amount of new contamination reaching the pasture, and the time relationships involved, appear to account adequately for the general trends of the tapeworms per day curve during the grazing seasons. They do not account for the absence of infection during the 1933-34 winter, nor for its lateness in the spring of 1934 as compared to the spring of 1933. The winter gap constitutes a good check on Daubney's (1932) observation that infection with this parasite is due to grazing. By November

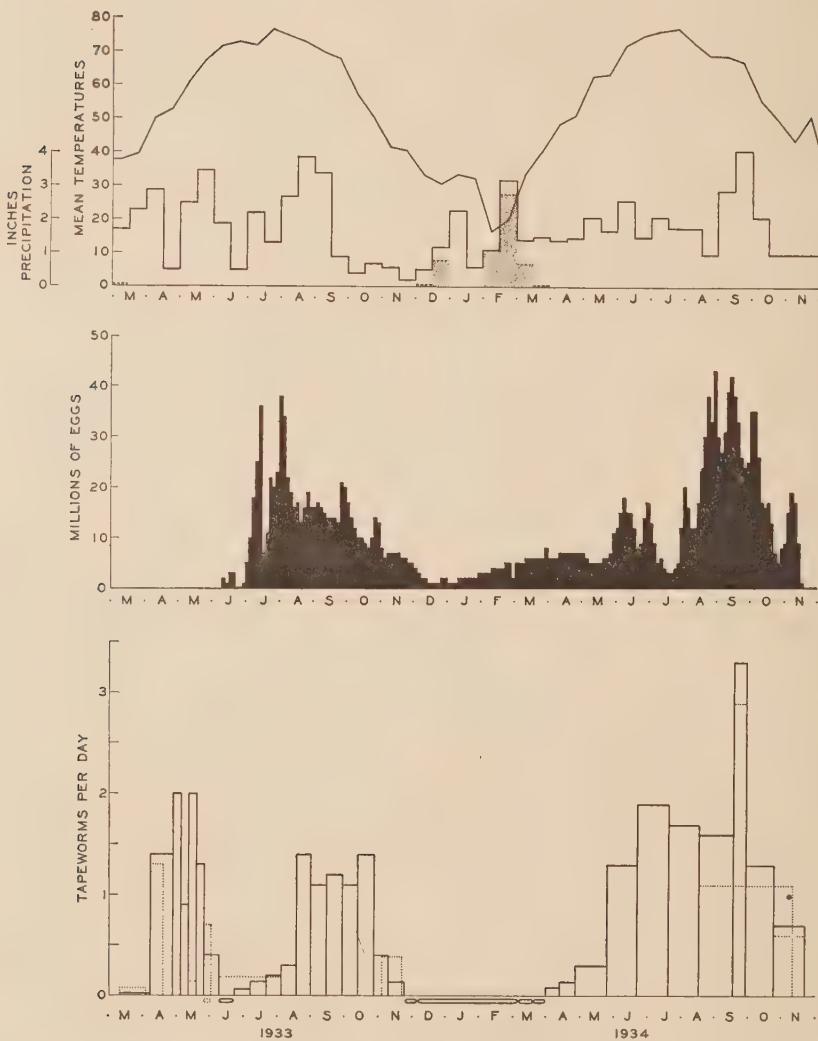


FIG. 4. The curve of tapeworms per day acquired by exposed susceptible hosts is here repeated (lower) as a measure of the infestation of the pasture, and shown in relation to the numbers of new tapeworm eggs which were reaching the area from all actively infected, i.e., patent, sheep (center graph). These millions of contaminating eggs have been calculated, for convenience in plotting, to 3-day periods.

To indicate the meteorological conditions under which *M. expansa* endemicity was maintained, precipitation and mean temperature data are given (upper) on a semi-monthly scale. Stippled portions represent snowfall, with 1 inch precipitation = 10 inches snow.

It is readily noted that the exhaustion phase in the pasture infestation during the late spring of 1933 is correlated with absence of early spring contamination. (Compare 1934.) New pasture infestation with *M. expansa* is reestablished in 1933 only after contaminating tapeworm eggs have been in the area for several weeks. Pronounced changes in the middle curve tend to anticipate changes in the lower, actual grazing seasons being considered. (See text.)

forage had become scanty in the pasture, and as it decreased in availability hay was supplemented. It was this withdrawal of the flock from actual grazing, as earlier noted, which was correlated with acquisition of a decreasing number of parasites by the sheep. With gradual re-establishment of good pasturage in the spring, feeding of hay was reduced and later stopped. This partly explains also the lateness of the area being demonstrably infested in early 1934 as compared with 1933.

At the top of figure 4 are the curves of mean temperature and precipitation by half-monthly intervals as obtained at the laboratory March, 1933–November, 1934. These show the range of climatic factors within which *Moniezia* endemicity was maintained. Also that there was no constant correlation between rainfall, or temperature, and the changes in the curve of tapeworms per day. The winter conditions intervening between the last observed new tapeworm contamination in the pasture in November, 1932, (Cf. Stoll, 1935b) and March, 1933, were not unusual, with mean monthly temperatures of 42.6, 36.1, 39.0 and 34.0 F, and precipitation normal, including snow in December and February of 8 and 13 inches respectively. These are in contrast to the sharply subnormal temperatures and excessive snow in the 1933–34 winter (especially in February, 1934), a combination of events which was reflected in a marked retardation of pasture development in the spring of 1934, as compared to the spring of 1933. Not only were normal grazing conditions late, but this was combined with the fact that the sheep, as earlier mentioned, were given an accessory pasture in addition to the run of the original field I–II. In these circumstances normal infection conditions for tapeworms were retarded for nearly two months, but once established were not significantly changed for the balance of the 1934 grazing season, except for one brief period of enhanced infectivity.

#### DISCUSSION

Two pictures are presented by the data in this 21-month study. One, derived primarily from fecal examination data of animals exposed in pasture long enough to show *M. expansa*, is that the area was continuously infested throughout each grazing season. The second, which takes account not only of the fact of infection, but also of its amount, indicates a cyclical condition in the infestation of the pasture. This was related to the recency, and also to the amount, of new contamination of the pasture by actively infected (patent) animals. In the absence of such new infecting material, the actual pasture infestation early in one season became depleted to practically a negative status; another season, by reason of uninterrupted contamination, the temporary exhaustion phase did not appear at all. The evidence is clear that overwintering is not a necessary factor in establishing infestation in an area.

Absence of infection during the winter period is ascribable to the fact that the animals were insulated from contact with the pasture "floor" by two factors: they were subsisting primarily on hay, and not grazing; and the pasture itself was snow-covered and frozen during part of the time. A delay in demonstrating the infested state of the pasture the second season of study, as compared to the first, is explainable as due to the delayed development of spring grazing which followed a severe winter, and accessibility by the sheep to new pasture land. That insulation from the pasture "floor" under winter conditions prevents infection was illustrated by an animal earlier reported (Stoll, 1936a). Placed in a 10-foot-square fenced enclosure of the pasture for one day only, December 28, 1934 (the month after the end of the period here under study) no infection resulted; placed in the same enclosure 12 days later after the dry grass had been raked so that the underlying greener grass would be attractive to eat, this sheep secured one tapeworm.

Rates of infection by sheep in the pasture were never very large, on a daily basis usually ranging from one to two tapeworms during most of each grazing season. Actual securing by sheep of but one or two tapeworms at a time is well attested. Besides the case just noted, another animal (No. 582, fig. 1) which was exposed in field II for just one day, November 7, 1934, secured just one tapeworm (Stoll, 1936a). This was near the end of the present study during a time when twin animals, simultaneously exposed, secured an average of 0.6 and 0.7 tapeworms daily for effective exposure periods of 29 days each. Two other cases of single *M. expansa* have been given, for a lamb in field I (Stoll, 1935b) and a calf (Stoll, 1937a) in field I-II. In the 1933-34 series four animals brought to autopsy were each parasitized by but one tapeworm (Figs. 1a and 1b). Besides the above we have had nine additional instances of infection by a single tapeworm as proved by autopsy. In all of these 17 cases, when the animals had been exposed longer than a day, it had been during periods of low pasture infestation. Brade-Birks (1927) in southeastern England found 9 of 19 slaughter-house lambs which were positive for *Moniezia* sp. possessed only one specimen each; Mönnig (1929) in South Africa recovered only one *M. expansa*, immature, from a 44-day lamb; Seddon (1931) in Australia determined that a lamb which can be calculated to have become infected its third week on pasture, had but one *M. expansa* when killed about 10 weeks later.

Just as lambs can be shown to contract but one tapeworm at a time, instances of only two parasites are likewise demonstrable. Four such occurrences in our 1933-34 series showed also an additional fact of interest, namely, that the worms in each such infection were of comparable size (respectively in cm: 1 and 1, 5 and 7, 18 and 23, 52 and 57), and thus presumably reached the host at or about the same time. In

another similar case (Stoll, 1937c) the two specimens were 4.5 and 5.0 meters long.

Evidence is thus abundant that *M. expansa* infections can occur one or two at a time, and the low daily rates demonstrated in the present study reflect a clearly-defined fact in the epidemiology of this species. The strobilate lengths in larger infections where animals have been exposed for a period of several weeks and the host at autopsy harbors many immature forms, show the gradation in size which further supports this conclusion, as Jenkins (1924) also points out. On the other hand, very large infections occurring at one time have not been demonstrable, and there is thus no support of Baer's (1927) hypothesis that asexual reproduction of larval stages may be needed to account for them. The highest rate in this study, 2.9 and 3.3 daily in twin animals simultaneously exposed, came a few weeks after excessively large depositions of new tapeworm eggs in the pasture. We have not observed pasture rates in excess of this, although in a small experimental enclosure, it was possible to build up the exceptional rate in a sheep of nearly 7 worms daily for 10 days of exposure.

The largest number of tapeworms found in any one animal during the 1933-34 study was 95. On only two occasions in field I-II have larger infections been recorded. In June to October, 1931, two bottle-raised spring lambs which died with heavy nematode infections showed respectively 109 and 241 *M. expansa*. While the latter is the largest number so far obtained from one host at this laboratory, the rates for these two animals were 1.3 and 2.9 tapeworms contracted daily, and thus in the range of the present study. A great many of the strobilae in both these cases were small—less than 1 cm.

The literature has frequent references to "numerous tapeworms" found in sheep, but actual counts of large numbers in a single host are infrequent. Leuckart (1885) refers to sheep infected with 50 or more scoleces, and Curtice (1892) notes "the number of individuals may be from 2 or 3 to a hundred, but it is unusual to find more than a half-dozen adults together." In Wales several observers have made counts of scoleces in abattoir animals, chiefly lambs. The largest number found in one host by Jones (1926) was 43, by Flattely (1922) 75, by Lewis (1930) 116 in each of two lambs, by Jenkins (1924) 230, and by Morgan (1923) 336. Morgan's is the highest count that has been encountered in the literature, and the worms showed various stages of growth, while in Jenkins' case measurements from a few millimeters to 240 cm are given for the tapeworms recovered. Brade-Birks (1927) in southeastern England noted a highest count of 79. Details relating to the infection conditions of slaughter-house animals are, of course, wanting, and the exact species of the tapeworms found in the above cases was not ascertained,

although they were all *Moniezia* and may be assumed to have been predominantly, if not exclusively, *M. expansa*. In Australia, Seddon (1931) had two lambs each showing 66 *M. expansa* at autopsy. These had effective exposures of 35 and 51 days, and thus contracted 1.6 and 1.3 tapeworms daily, a figure within the range of our present study. Insofar as these data can be interpreted, therefore, they emphasize our conclusion that a prime characteristic of *M. expansa* infection is the promptness and regularity with which sheep grazing in infested fields contract this cestode in relation to the amount of infestation present (Stoll, 1937d). The rule with *Moniezia* is not the securing of large numbers of tapeworms at one time.

There is one sense in which the state of pasture infestation revealed in our study may not be typical. If a flock with spring lambs is turned into a pasture early in the spring, many of the lambs would become infected at about the same time and consequently contaminate the area with an excessively large number of new tapeworm eggs. After a lag of a few weeks these would establish such reinfestation of the pasture as would probably serve to hasten the immunization of members of the same flock, and would in part presumably survive to the following season. On the other hand, other studies, as well as our own, have shown the semi-sterilizing effect of dry seasons on the pasture infestation (Mönning, 1929; Stoll, 1935b). Under such conditions concentration of new contamination over a limited period of weeks might permit very large losses in the pasture tapeworm population, larger than might have occurred in such conditions as prevail in our study, where for a year and a half the area was constantly receiving new tapeworm eggs under favorable conditions of temperature and rainfall. In this connection two points of interest are apparent in figures 1 and 3. The first is the notable delay with which pasture-born lambs contract the tapeworms, to which attention has earlier been called (Stoll, 1937c). The other is the marked exhaustion of the pasture infestation in the absence of new contamination early in the 1933 grazing season. To the extent that a pasture was being grazed over by older immune sheep this exhaustion of the infestation through its destruction by such animals might well exercise a distinct controlling effect on the infection of susceptible lambs later.

This study was completed nearly three years before Stunkard (1937a, b, c) announced larval stages of *M. expansa* developing in oribatid mites. If ingestion of live beetle mites containing matured cysticercoids is the method by which sheep infection with this tapeworm normally occurs, then the present study reveals certain characteristics of the intermediate host in this region. These include infection of sheep in the early spring by overwintering mites that are already infected; almost complete depletion by June of the overwintering mite population in a pasture where

sheep are grazing freely; the common occurrence in the intermediate host of only one or two cysticercoids which infect; and a required period for development of cysticercoids in the mites which is not in excess of 2 months under summer pasture conditions, and may be half that. While definitive data are still meager concerning the rôle of the mite, on comparative grounds such rôle should be favored, even though it is obvious that the epidemiological facts presented are essentially harmonious with a conception of *Moniezia* embryos maturing within the eggs after a manner comparable to the development of infectivity of *Capillaria* or *Trichuris* eggs.

Perhaps one of our observations definitely suggests some intermediate host as the method of infecting the sheep. In infections where only two tapeworms were found, each of our five such cases showed tapeworms of practically identical size.

#### SUMMARY

The changing state of pasture infestation with *Moniezia expansa* has been followed by determining the daily infection rates of a succession of sheep and lambs allowed to graze in the area for limited periods, and examining them post mortem for the number of tapeworms they had contracted. Control animals were exposed in the test area at the same time and their infections dated by when they became positive for the tapeworm at fecal examination. Altogether 21 consecutive months, including two grazing seasons and the intervening winter, were under observation. Neither age of susceptible hosts, nor concentration of sheep per acre of pasture, could be shown influential in modifying the number of tapeworms contracted. Absence of grazing during the winter months when hay was fed did prevent infection. At the beginning of one grazing season there occurred an initial increase in the infested state of the pasture followed by gradual exhaustion of the infestation in the absence of new contamination of *Moniezia* eggs from actively infected (patent) animals. Pasture infestation was not restored immediately after such contamination, but only following a lag period of a few weeks. To a striking degree changes in the gross number of new contaminating eggs in the area anticipated changes in the degree of the ensuing pasture infestation.

In general, the number of *M. expansa* acquired by susceptible sheep was low, being one to two tapeworms daily during most of the grazing season. Numerous instances of infection with only a single tapeworm were demonstrated, these occurring after short periods of grazing (one day), or after longer periods when the pasture infestation was low. In a small series of cases in which but two tapeworms were contracted by the hosts, the strobilae were of substantially the same size. This fact, that the nature of the pasture infestation is such as to permit not only low

daily rates of acquiring the tapeworms, but the actual securing of infections limited to but one or two tapeworms by individual hosts, is one of the striking features of *Moniezia* epidemiology.

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Postscript added in proof: In view of the results of the variation in pasture infestation it is of interest to be able to add at this time a report of the development of cysticercoids in oribatid mites following their evident ingestion of *M. expansa* eggs in the laboratory, and the infection of sheep with tapeworms after feeding mites to them. No infection of susceptible sheep was secured either by feeding apparently mature cysticercoids freed from mites 6 weeks after exposure to the eggs, or by feeding live mites from such positive batches. (In an unreported field experiment, a sheep, grazing in a hitherto uninfested enclosure, contracted *M. expansa* 24–28 days after initial contamination of the area with tapeworm eggs; this interval is calculated from first appearance of segments in the patent animal 35–38 days later.) Several sheep have been fed mites which had been exposed to tapeworm eggs for longer periods. In two cases these hosts were successfully infected. In one instance a delayed patent infection resulted in a yearling ram after feeding mites which had been first exposed to eggs 10–11 weeks earlier, but had not been in contact with eggs for 6–8 weeks when administered to the sheep. A single mature tapeworm, confirmed as *M. expansa*, was recovered at autopsy of the host; feeding of eggs from its gravid segments to *Galumna* sp. and *Galumna nigra* (Ewing) resulted in infection of the mites, cysticercoids being demonstrable in 6–7 weeks. (*Ceratozetes* sp. and *Oribatella magniseta* Ewing fed and examined with the *Galumna* were negative.) In a second instance, a single specimen 3.6 cm long was recovered from a yearling wether killed 14 days after feeding to it mites experimentally exposed to tapeworm eggs for a period of 2–4 weeks, beginning 9–11 weeks before administration to the sheep; this strobilate length fits observations noted in Stoll, 1937c. In each of these two infections the mites had died before being fed. Only *M. expansa* was used, and in all cases the sheep employed were of known parasitic history and previously uninfected. The observations are considered confirmatory of Stunkard, 1937a and b, on the development of *Moniezia* cysticercoids in oribatid mites, and of the added note in Stunkard, 1937c, on infection of sheep by infected mites. I am indebted to Dr. H. E. Ewing for the mite identifications.—N. R. S.



ISOSPORA BOUGHTONI N. SP. FROM THE AMERICAN  
OPOSSUM, *DIDELPHIS VIRGINIANA*

JOSEPH J. VOLK

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Examinations of the opossum, *Didelphis virginiana*, from the vicinity of Athens, Georgia, have shown them to be parasitized by an undescribed coccidian of the genus *Isospora*. The only coccidian reported in the literature from a marsupialian host was named *Eimeria macropodis* by Wenyon and Scott in 1925 from Bennett's wallaby, *Macropus bennetti*, in the Zoological Gardens of London, England.

In one opossum minutely examined, the upper small intestine for a length of four inches below the stomach was filled with a bloody mass containing numerous fully-sporulated oocysts. There was a very heavy infection of gametocytes, schizonts and fully-sporulated oocysts, each developing above the nucleus of its host-cell in the epithelial and sub-epithelial tissues. The outer cells of the villi were sloughed off into the lumen of the intestine.

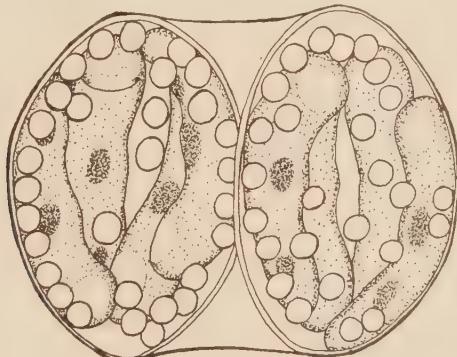


FIG. 1. Sporulated Oocyst of *Isospora boughtoni* n. sp.

The oocyst was of irregular shape due to the extremely delicate wall tightly enclosing the two ovoid sporocysts. Although the oocysts were merely two sporocysts enclosed in a thin oocyst wall and inconstant in position, their measurements are given for convenience:  $16.8-20.4 \mu \times 10.8-12.0 \mu$ . There was no micropyle. There was no residual body in the oocyst, which differentiates it from the common tissue-sporulating types of *Isospora* reported for carnivores. The sporocysts, measuring  $8.4-9.6 \mu \times 12.0-13.2 \mu$ , mean  $8.93 \times 12.24 \mu$ , form-index 0.72, each contained numerous round, hyaline-refractile inclusion bodies measuring

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about 1  $\mu$  in diameter. There was no "plug" in the sporocyst wall. The sporozoites were somewhat sausage-shaped, tapering at one end, with a hyaline body at the opposite end. In prepared sections there appeared three deeply-staining bodies in the sporozoites: the hyaline body at the blunt end, the nucleus in the middle, and a body, similar to the nucleus, near the more pointed end.

This *Isospora* bears a similarity in oocyst appearance to that pictured for *Isospora buteonis* Henry (1932) of the hawk and owl, which does not exist between it and the *Isospora* of carnivores. The sporocyst contains numerous hyaline bodies, while those of carnivores have a distinctive, large granular residual body. No *Isospora* has been reported from this type of host. In addition, the third staining body in the sporozoites is not to be seen in other *Isospora*. The name *Isospora boughtoni* n. sp. is proposed in honor of Dr. Donald C. Boughton, to whom the writer is greatly indebted.

*Isospora boughtoni* n. sp.

(Fig. 1)

*Specific diagnosis:* COCCIIDA, EIMERIIDAE. Genus *Isospora*. Characteristics of the genus. The oocyst produces two spores, each containing four sporozoites. Oocysts irregularly shaped; no micropyle; no residual body; wall delicate; tissue-sporulating; measurements on 25 oocysts: 16.8–20.4  $\mu$   $\times$  10.8–12.0  $\mu$ ; mean 15.6  $\times$  11.2  $\mu$ .

Sporocysts ovoid, without polar differentiation; numerous hyaline-refractile inclusions; range 8.4–9.6  $\mu$   $\times$  12.0–13.2  $\mu$ ; mean 8.9  $\times$  12.2  $\mu$ . Sporozoites sausage-shaped, each with three deeply staining bodies; size 2.4  $\times$  8.3  $\mu$ .

*Host:* *Didelphis virginiana* (MAMMALIA, MARSUPIALIA). Opossum.

*Habitat:* Epithelium and subepithelium of upper small intestine.

*Locality:* Athens, Georgia.

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WENYON, C. M. AND SCOTT, H. H. 1925 Demonstrations of sections of intestine of Bennett's Wallaby, *Macropus bennetti*. *Tr. Roy. Soc. Trop. Med. and Hyg.* **19**: 7.

## RESEARCH NOTES

### ON THE MOTHER REDIA OF *DIPLODISCUS TEMPERATUS* STAFFORD, 1905

Among the life cycle descriptions of amphistomes we find some with one and others with two generations of rediae. Looss (1892, *Festschr. Leuckart*, pp. 147-167) reported one generation of rediae for *Amphistomum subclavatum* and (1896, *Recherches sur la faune parasitaire de l'Egypte*) two generations for *Paramphistomum cervi* (syn. *Amphistoma conicum*) and *Gastrodiscus aegypticus*. Beaver (1929, *J. Parasitol.* 16: 13-23) described two generations of rediae for *Allassostoma parvum*. Le Roux (1930, 16th Rept. Dir. Vet. Serv., Dept. Agric. Union S. Africa, Pretoria, pp. 243-253) mentioned the fact that mother rediae were present in the life cycle of *Cotylophoron cotylophorum* and Bennett (1936, *Illinois Biol. Monogr.* 14: 1-119) described both generations of rediae for the same species. Krull and Price (1932, *Occas. Papers Mus. Zool., Univ. Mich.* No. 237, pp. 1-38) reported only one generation for *Diplodiscus temperatus*. In this note a first generation of rediae is described for *D. temperatus*.

First generation rediae were found on July 24, 1935, and July 22, 1938. Adult cercariae were emerging from the snails containing them. Only three of these rediae were recovered from among the great number of cercariae-producing rediae. In one of these rediae (Fig. 1) the anterior region was almost continu-

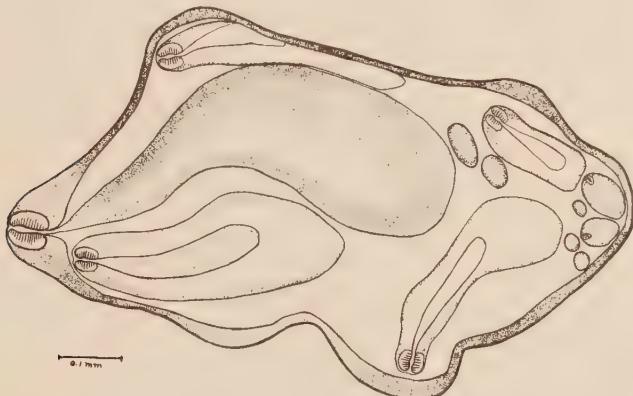


FIG. 1. Mother redia of *Diplodiscus temperatus*.

ally in motion. Two pairs of appendages similar to those described for daughter rediae of this species are found in mother rediae. Definite annulations are seen on the body surface and appendages. The length of the two rediae found in 1938 measure respectively 0.85 and 0.91 mm. At the anterior end is located the large and almost round pharynx. The pharynx in the largest specimen measures 54 by 47  $\mu$  and in the smaller one 44 by 44  $\mu$ . The esophagus is short and enlarges into an intestine which extended in one specimen to the region of the anterior appendages. In another specimen the intestine was squeezed by the daughter rediae to extend to the region of the posterior appendages. One large mother redia was distinguished from the mass of other rediae by the motion of the brownish intestinal contents of one daughter redia.

Rediae and cercariae were not observed developing together in any of these rediae.—E. C. HERBER, *Biology Department, Dickinson College, Carlisle, Pa.*

THE EFFECT OF INFECTION WITH *DAVAINEA PROGLOTTINA* ON THE WEIGHTS OF GROWING CHICKENS

Although numerous instances of the pathogenicity of the poultry cestode, *Davainea proglottina*, have been reported, all of these observations have been made on naturally infected birds under field conditions. Taylor (1933, Proc. 5th World's Poultry Congress 3: 219) reported that 10-week-old chickens experimentally infected with large numbers of *D. proglottina* did not show any ill effects or perceptible weight losses as a result of the parasitism. It appears that no one has ever reported harmful effects of this parasite under controlled experimental conditions.

This paper gives the results of an experiment to determine the effect of an experimentally produced infection with *D. proglottina* on the weights of growing chickens. Thirty-four parasite-free single comb white Leghorns, seven weeks old, were divided into two groups of seventeen each. To facilitate the statistical analysis of the weight records, each bird in one group was matched with a bird of the same sex and weight in the other. Statistical analyses of the data were made by the method of Fisher (see Levine, 1938, J. Parasitol. 24: 45).

Approximately seventy-five slugs (*Agriolimax agrestis*), experimentally infected with cysticercoids of *D. proglottina*, were fed to each bird in one group. Half of the slugs were fed during the first three weeks of the experiment and the remainder were fed during the following two months. The second group was not infected and served as a control. Both groups were held under identical conditions in batteries with wire screen bottoms. Feed and water were kept before them at all times. No adventitious parasitisms were encountered during the experiment.

The birds were weighed at frequent intervals and the weights are recorded in Table 1. All differences between mean weights (except days 1 and 26) were statistically significant, standard errors being 0.22 to 0.42 times mean weight difference. On the 17th day of the experiment the mean weight of the infected birds was significantly greater than that of the controls. The reason for this is not clear. The weight of the controls was greater than that of the infected group beginning with the 35th day and continuing throughout the duration of the experiment (136 days). The difference in weight between the two groups gradually increased until a maximum of 6 ounces was reached on the 88th and 101st days. Although the weight difference afterwards gradually decreased, it was always significantly in favor of the non-parasitized controls. It will be noted that the weights of both groups, which had been increasing steadily from the beginning of the experiment, dropped on the 101st day. This was due to an outbreak of an acute respiratory infection which affected all the birds. Recovery from this infection was complete 10 days later.

Food consumption by each group up to the 101st day was practically identical, after which records were not kept.

Symptoms that could be directly attributed to the parasitism were not noted. During the course of the experiment three infected birds and one control had to be eliminated due to extraneous conditions. Whenever a bird in either group was eliminated the bird with which it was paired was taken out also. The number of scoleces found in the intestines of the three infected birds was 5617, 2595, and 1932 respectively.

It should be noted that the difference in weight between the infected and the control group was not only statistically significant but also significant from a practical point of view. The weight of birds 13 weeks after infection and then 20 weeks old was six ounces (about 12 per cent) less than that of the controls. Insofar as the writer can ascertain, this is the first time that the unfavorable effect of tapeworms on chickens has been demonstrated experimentally.—P. P. LEVINE, New York State Veterinary College, Ithaca, New York.

TABLE 1.—Effect of infection with *Davainea* protozoa on the weight, in ounces, of growing chickens.  
(Experimental infections at seven weeks of age)

Number Days on Experiment.....	1	17	26	35	42	49	58	65	72	79	88	101	109	115	123	129	136
Number Birds in Each Group .....	17	17	17	17	17	16	14	14	14	14	14	13	13	13	13	13	13
Mean Weight of Control Birds.....	9	14	20	26	30	34	39	42	46	47	53	50	50	52	54	56	58
Mean Weight of Infected Birds.....	9	15	20	24	27	30	35	38	41	42	47	44	45	47	50	52	54
Greater Mean Weight of Control Birds .....	0	-1	0	2	3	4	4	4	5	5	6	5	5	4	4	4	4

## A SURVEY OF THE INTESTINAL PARASITES OF MEDICAL STUDENTS

This paper reports the results of an examination of fecal samples from 291 medical students to determine the incidence of intestinal parasites. The survey was undertaken as a part of a study of the epidemiology of amebiasis in various groups of the population of New Orleans. This group includes all four classes of the student body of the Louisiana State University School of Medicine and consists almost entirely of males ranging from 21 to 27 years of age.

A single fecal sample from each student was examined, except when it was impossible to make an accurate diagnosis, and in this case a repeat specimen was requested. Only fresh samples, usually received within 12 hours after passage and obtained without catharsis, were examined. Five different technics were employed in the examination of each sample, making a total of 1455 examinations. The following methods were used for each specimen: (1) an aqueous plain smear with an unstained and iodine-stained portion; (2) a centrifuged specimen which was washed twice; (3) brine centrifugal flotation; (4) a sedimented specimen which was repeatedly washed and allowed to settle until the supernatant fluid was clear; (5) a zinc sulphate flotation technic which was devised by Faust and coworkers (1938, Am. J. Trop. Med. 18: 169). The latter method employs the principle of levitation, as in brine flotation, but differs from it in that zinc sulphate is substituted for sodium chloride to produce a specific gravity of 1.180. It is important to note that this method isolates and concentrates both protozoan cysts and helminth ova from the stool specimen.

*Results.*—The combined results of all five methods revealed that of the 291 individuals, 85 or 29 per cent were positive for either pathogenic or non-pathogenic parasites. The examination revealed 61 individuals (21 per cent) with a single infection, 21 (or 7 per cent) harboring two infections, and 3 (or 1 per cent) with three infections. Thirty individuals (10 per cent of the entire group) harbored parasites, including *E. histolytica*, *N. americanus* and *S. stercoralis*, which are definitely considered pathogenic.

TABLE 1.—*The incidence of the various intestinal parasites*

	Number positive					Per cent positive
	Fresh-men	Sopho-mores	Juniors	Seniors	All classes	
<i>E. histolytica</i> .	3	2	2	2	9	3
<i>E. nana</i> .....	13	6	11	5	35	12
<i>E. coli</i> .....	9	7	4	10	30	10
<i>I. butschlii</i> ..	2	0	1	0	3	1
<i>G. lamblia</i> .....	9	1	2	0	12	4
<i>N. americanus</i> .	5	5	3	1	14	5
<i>S. stercoralis</i> .	2	0	3	1	6	2
<i>T. trichiurus</i> .	0	1	0	0	1	0.3
Mites .....	0	2	0	0	2	0.7
Positive stools.	32	21	18	14	85	
Total examined (with per cent positive) .....	92 (33%)	78 (27%)	59 (31%)	62 (23%)	291 (29%)	

In the above table the medical students are divided into four class groups because the junior and senior students had been examined for intestinal parasites one and two years, respectively, prior to this survey, and those individuals found positive for *E. histolytica* had been carefully treated for the infection. Thus, examination of the table calls attention to the fact that in these two classes at the time this survey was carried out, the incidence of *E. histolytica* was approximately the same as in the other two classes, which had not been previously ex-

amined or treated. Although only very fresh specimens were examined and over 150 of the 291 specimens were cultured on Cleveland's *Entamoeba* medium, *Dientamoeba fragilis* was not observed in any case.—J. C. SWARTZWELDER, Department of Preventive Medicine and Public Health, Louisiana State University, School of Medicine, New Orleans, La.

**TRICHOMONAS GALLINAE (RIVOLTA, 1878) THE CORRECT NAME FOR THE FLAGELLATE IN THE MOUTH, CROP AND LIVER OF THE PIGEON**

Much confusion exists regarding the name of the *Trichomonas* from the upper digestive tract and liver of the pigeon. It is generally agreed that Rivolta first saw and described this parasite, but the exact name and date are matters of dispute. Having gone over the situation, it was felt that the results might justify their publication.

The writer is indebted to the reference librarians of the university for their part in tracing down Rivolta's early papers and is especially grateful to Miss Catherine Marasco of this department for translating the original Italian texts.

It appears that Rivolta did not describe *T. columbae* in 1878. This name was introduced in a work on the diseases of domestic and semi-domestic fowl in 1880 (some give 1881) by Rivolta and Delprato (Pisa) for a flagellate in the small intestine of the pigeon. The writer has been unable to procure this publication and takes his information in part from von Rátz (1913, *Centr. Bakt. I Abt. Orig. 71*: 184-189). Cauthen (1936, *Am. J. Hyg.* **23**: 132-142) has likewise found flagellates in the small intestines of pigeons and he identifies them with the *Trichomonas* from the crop, which appears to be able to pass into the post-gastric intestine under certain conditions.

In 1878, however, Rivolta did describe *Cercomonas gallinae* from the mouth and crop of a pigeon and *C. hepaticum* from the liver (1878, *G. Anat., Fisiol. e Patol. Anim.* **10**: 149-158). Nieschulz and Bos (1936, *Centr. Bakt. I Abt. Orig. 135*: 473-475) recognized the priority of the 1878 names over *T. columbae*, yet went astray on the *C. gallinae*-*C. hepaticum* situation, stating that the organism should be called *T. hepatica*.

Reviewing Rivolta's 1878 paper in detail we see that he published what might be considered either as three separate papers or as a single one. Pages 149-154 discuss "Una forma di croup prodotta da un infusorio, nei pollii," the title being followed by Rivolta's name, etc. This ends on page 154 and, immediately following, to 157, is considered "Una specie di epatite caseosa prodotta da un infusorio nel piccione." On page 157 is another title, "Numerose macchie per infiltrazione granulo bacteriosa sulla mucosa intestinale di un piccione nidiace." Though Rivolta is obviously the author of all three, neither the second nor the third title bears his name. Also, in the journal index these three are listed as separate items.

In his first discussion (pp. 149-154) Rivolta describes two things; a patchy, caseous involvement of the mucosa of the upper digestive tract of a pullet and a yellowish, scabby, patchy coating involving the mouth, esophagus and crop of a pigeon. In the chicken he found no causative agent, whereas in the pigeon he reports (as translated) "thousands and thousands of infusoria in form of round cells, of which many moved with a truly surprising rapidness." These organisms from the pigeon, undoubtedly what we are calling *T. columbae* today, he named *Cercomonas gallinae*.

In the second section, that on the caseous pigeon hepatitis (pp. 154-157), Rivolta again described a flagellate as the cause of the disease, calling it *Cercomonas hepaticum*. This, too, was probably the so-called *T. columbae*, as both Oguma (1931, *J. Fac. Sc., Hokkaido Imp. U., Series 6, 1*: 117-131) and Cauthen (1936, *Am. J. Hyg.* **23**: 132-142) have recently described a lethal hepatitis in pigeons and doves caused by this organism. The third section (pp. 157-158) deals with bacteria and need not concern us. We see that at no time in these pages did Rivolta mention the name "columbae."

It is clear then, that Rivolta in 1878 gave two names to what was the same flagellate from different regions in different pigeons. Therefore, as only one type of flagellate has been described from the mouth, crop or liver lesions of pigeons and as Rivolta first called this organism *Cercomonas gallinae* in 1878, there is no justification for the revival by Nieschulz and Bos (1936) of *C. hepaticum*, or for the use of *T. columbae* Rivolta and Delprato (1880), which likewise becomes a synonym of *Trichomonas gallinae* (Rivolta, 1878).—ROBERT M. STABLER, Department of Zoölogy, University of Pennsylvania.

#### TRICHOMONAD FLAGELLATES IN FACIAL LESIONS OF A PIG

Several species of *Trichomonas* appear to be pathogenic to their hosts, for example, *T. columbae* of pigeons and doves and *T. foetus* of cattle, and others are of doubtful pathogenicity, hence the discovery of trichomonad flagellates in facial lesions of unknown origin in a pig seem worth recording.

The pig, a pure bred Tamworth, was born in Honolulu, Hawaii, on June 30, 1937, and was obtained from the University herd through Dr. S. H. Work. Its "wolf" teeth were cut at the age of one week. Soon after weaning (Aug. 30), the animal developed scours; on about Oct. 1, a swelling was noted on the left side of the face; corresponding swellings were noted on the right side of the upper jaw and in the region of the canine teeth of the lower jaw. Some nasal discharge and sneezing were also observed. On Oct. 30, 1937, the pig was killed. The swelling on the right side had broken on the outside, and a large crust was noted on the skin. Sections of these enlargements revealed a thickened skin and necrosis of the underlying tissues extending close to the facial bones. Smears made of the necrotic tissue revealed many trichomonad flagellates. In a preliminary bacteriological examination of the necrotic tissue by Dr. O. N. Allen, of the Bacteriology Department, University of Hawaii, *Pseudomonas pyocyaneus* and *Actinomyces* were found.

Smears were stained with iron hematoxylin and ten typical specimens were measured. They ranged in length from 7 to 15  $\mu$  and in breadth from 4 to 7  $\mu$ . Their average length was 10.3  $\mu$  and average breadth 5.6  $\mu$ . Sections were cut of the tissue but no trichomonads were identified with certainty among or within the cells. The pathogenicity of these trichomonads is thus uncertain. The possibility is suggested that flagellates living in the mouth of the pig invaded the interstices of the necrotic tissue where they encountered favorable conditions for growth and reproduction.—ROBERT HEGNER, Department of Protozoology, School of Hygiene and Public Health, Johns Hopkins University, AND J. E. ALICATA, University of Hawaii.

#### OOCHORISTICA PARVULA N. NOM. FOR OOCHORISTICA PARVA STUNKARD, 1938, PREOCCUPIED

In a recent paper (1938 Carnegie Inst. Wash. Publ. No. 491, pp. 33-50) Stunkard described a new cestode from *Coleonyx elegans* as *Oochoristica parva*. Since the name *O. parva* is preoccupied by *O. parva* (Janicki, 1904) Baer, 1935 (vide Baer, 1935, Rev. Suisse Zool. 42: 278) a new name, *Oochoristica parvula*, is proposed for the species.—HORACE W. STUNKARD, New York University.

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## PROGRAM AND ABSTRACTS OF THE FOURTEENTH ANNUAL MEETING OF THE AMERICAN SOCIETY OF PARASITOLOGISTS

RICHMOND, VIRGINIA

DECEMBER 28, 29 and 30, 1938

### PROGRAM<sup>1</sup>

WEDNESDAY MORNING SESSION, DECEMBER 28, 10:00 A. M.; ROOM 209, MCGUIRE  
HALL, MEDICAL COLLEGE OF VIRGINIA.

#### *Read*

1. Balamidiasis. (15 min) (Lantern) MARTIN D. YOUNG, U. S. Public Health Service, Columbia, S. C.
2. The Results of a Six-year Protozoological Survey of Food Handlers in a Collegiate Institution. (10 min) (Lantern) D. H. WENRICH AND JOHN H. ARNETT, University of Pennsylvania.
3. Comparative Efficiency of Various Technics for the Discovery of Protozoa and Helminths in Feces. (15 min) (Lantern) E. C. FAUST, W. SAWITZ, J. TOBIE, V. ODOM, C. PERES AND D. LINCICOME, National Institute of Health and Tulane University.
4. Hematoxylin Staining of Protozoan Cysts Obtained from Feces by Zinc Sulphate Levitation. (5 min) (Also by demonstration) HANNAH S. NICKEL, Mississippi State Hygienic Laboratory, National Institute of Health and Tulane University.
5. The Presence of Pinworm (*Enterobius vermicularis*) Ova in Household Dust. (10 min) M. O. NOLAN AND LUCY REARDON, National Institute of Health.
6. Comparative Efficiency of the NIH Anal Swab Examination and Stool Examination by Brine and Zinc Sulphate Flotation for *Enterobius* Infection. (5 min) (Lantern) W. SAWITZ, V. ODOM AND D. LINCICOME, National Institute of Health and Tulane University.
7. The Treatment of Oxyuriasis with an Improved Type of Enteric-coated Tablet. (10 min) WILLARD H. WRIGHT AND FREDERICK J. BRADY, National Institute of Health.
8. *Bufo marinus* as a Vector of Helminth Ova in Puerto Rico. (10 min) (Lantern) W. A. HOFFMAN AND J. L. JANER, School of Tropical Medicine, San Juan, P. R.
9. Preliminary Report on the Incidence of Trichinosis in Alabama. (10 min) (Lantern) J. HENRY WALKER AND C. G. BRECKENRIDGE, University of Alabama.
10. Studies on the Protective Power of Serum from Dogs Actively Immunized against *Ancylostoma caninum*. (15 min) (Lantern) G. F. OTTO, Johns Hopkins University.

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<sup>1</sup> An alphabetical author index will be found at the end of the program.

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12. Schistosomiasis Due to *Schistosoma mansoni* and Its Transmission in Ethiopia. ARNALDO GIOVANNOLA, Italian Institute of Public Health, Rome, Italy.
13. Notes on the Incidence of Human Intestinal Helminths in the State of Cojedes, Venezuela, South America. WILLIAM HUGH HEADLEE, Purdue University.
14. The Possible Rôle of Birds in the Epidemiology of Sylvatic Plague. WILLIAM L. JELLISON, U. S. Public Health Service, Hamilton, Mont.
15. The Tick, *Ornithodoros hermsi*, as Probable Vector of Relapsing Fever in Northern Idaho. CORNELIUS B. PHILIP AND GORDON E. DAVIS, U. S. Public Health Service, Hamilton, Mont.

WEDNESDAY AFTERNOON SESSION, DECEMBER 28, 2:00 P. M.; ROOM 209, MCGUIRE HALL, MEDICAL COLLEGE OF VIRGINIA.

## Read

16. Blood Alterations in Typhlohepatitis of Turkeys, with Notes on the Disease. (12 min) E. P. JOHNSON AND C. J. LANGE, Virginia Agricultural Experiment Station, Blacksburg, Va. (Introduced by BRUCE D. REYNOLDS.)
17. Cultures of *Pentatrichomonas* sp. in vitro from the Livers of Chickens and Turkeys. (10 min) ENA A. ALLEN, U. S. Bureau of Animal Industry.
18. Domestic Fowls as Carriers of the Poultry Gapeworm. (10 min) EVERETT E. WEHR, U. S. Bureau of Animal Industry.
19. Goblet Cells and Age Resistance to Parasitism. (8 min) (Lantern) J. E. ACKERT AND S. A. EDGAR, Kansas State College.
20. Effects of the Tapeworm *Raillietina cesticillus* (Molin) on Growing Chickens. (7 min) J. E. ACKERT AND A. A. CASE, Kansas State College.
21. Susceptibility of Chickens to Reinfection with *Raillietina cesticillus* as Determined by the Presence of the Original Terminal Segment. (15 min) (Lantern) GEORGE W. LUTTERMOSER, U. S. Bureau of Animal Industry.
22. A Method for Testing the Rôle of Host Activity in Coccidian Periodicity. (7 min) (Lantern) DONALD C. BOUGHTON, University of Georgia.
23. Dried Milk Products in the Ration and Mortality from Cecal Coccidiosis in Chicks. (15 min) (Lantern) ELERY R. BECKER, Iowa State College.
24. Observations on *Eimeria bukidnonensis* in New York State Cattle. (10 min) (Lantern) DONALD W. BAKER, N. Y. State Veterinary College, Cornell University.
25. Fifteen Cases of Experimental Bovine Venereal Trichomoniasis. (15 min) (Lantern) (Also by demonstration) CHARLES W. REES, U. S. Bureau of Animal Industry.
26. Sterile Culture of the Free-living and Parasitic Larval Stages of *Haemonchus contortus*. (15 min) (Lantern) (Also by demonstration) R. W. GLASER AND NORMAN R. STOLL, Rockefeller Institute for Medical Research, Princeton, N. J.
27. The Efficacy of Phenothiazine for the Removal of Ascarids and Nodular Worms from Swine. (15 min) PAUL D. HARWOOD, A. C. JERSTAD AND LEONARD E. SWANSON, U. S. Bureau of Animal Industry.

## By Title

28. New Intermediate Hosts of the Fowl Tapeworm *Raillietina cesticillus* (Molin). A. A. CASE AND J. E. ACKERT, Kansas State College.
29. Barium Antimony Tartrate as a Remedy for the Removal of Gapeworms from Chickens. EVERETT E. WEHR, PAUL D. HARWOOD AND JACOB M. SCHAFER, U. S. Bureau of Animal Industry.

30. Hemorrhage as the Cause of the Fatal Anemia Associated with Stomach Worm Infection in Sheep. JOHN S. ANDREWS, U. S. Bureau of Animal Industry.

THURSDAY MORNING SESSION, DECEMBER 29, 9:30 A. M.; ROOM 209, MCGUIRE HALL, MEDICAL COLLEGE OF VIRGINIA.

*Read*

31. A Study of Periodicity and Synchronicity of the Pigeon Strain of *Plasmodium relictum*. (12 min) (Lantern) G. ROBERT COATNEY, U. S. Public Health Service, Columbia, S. C.

32. Cross Immunity Reactions in Avian Plasmodia. (10 min) (Lantern) FRUMA WOLFSON, Johns Hopkins University.

33. Studies on Immunity in Avian Malaria, with Special Reference to *Plasmodium circumflexum*. (12 min) (Lantern) REGINALD D. MANWELL AND FREDERICK GOLDSTEIN, Syracuse University.

34. Cellular Reactions to Malaria in the Skin of Normal and Immune Canaries and Monkeys. (15 min) (Lantern) WILLIAM H. TALIAFERRO AND WILLIAM BLOOM, University of Chicago.

35. Comparative Study of the Morphology and Life History of Two Species of Frog Filaria. (10 min) (Also by demonstration) O. R. CAUSEY, Johns Hopkins University.

36. The Relation of Shade to *Anopheles quadrimaculatus* Breeding. (12 min) (Lantern) E. HAROLD HINMAN AND HERBERT S. HURLBUT, Tennessee Valley Authority.

37. Acquired Immunity to Ticks. (15 min) (Lantern) WILLIAM TRAGER, Rockefeller Institute for Medical Research, Princeton, N. J.

THURSDAY MORNING, DECEMBER 29, 11:15 A. M.; ROOM 209, MCGUIRE HALL, MEDICAL COLLEGE OF VIRGINIA.

*Presidential Address*

38. Some Problems in Medical and Veterinary Entomology. F. C. BISHOPP, U. S. Bureau of Entomology and Plant Quarantine.

THURSDAY NOON, DECEMBER 29.

12:30 PARASITOLOGISTS' LUNCHEON, HOTEL RICHMOND, for members and guests.

1:30 ANNUAL BUSINESS MEETING.

THURSDAY AFTERNOON SESSION, DECEMBER 29, 3:00 P. M.; ROOMS 304 and 306, MCGUIRE HALL, MEDICAL COLLEGE OF VIRGINIA. (Tea will be served.)

*By Demonstration*

4. Hematoxylin Staining of Protozoan Cysts Obtained from Feces by Zinc Sulphate Levitation. (Also read) HANNAH S. NICKEL, Mississippi State Hygienic Laboratory, National Institute of Health, and Tulane University.

25. Fifteen Cases of Experimental Bovine Venereal Trichomoniasis. (Also read) CHARLES W. REES, U. S. Bureau of Animal Industry.

26. Sterile Culture of the Free-living and Parasitic Larval Stages of *Haemonchus contortus*. (Also read) R. W. GLASER AND NORMAN R. STOLL, Rockefeller Institute for Medical Research, Princeton, N. J.

35. Comparative Study of the Morphology and Life History of Two Species of Frog Filaria. (Also read) O. R. CAUSEY, Johns Hopkins University.

39. The Localization of Glycogen in *Macracanthorhynchus hirudinaceus*. THEODOR VON BRAND, Barat College of the Sacred Heart, Lake Forest, Ill.

40. Hemoglobin in Turtle Parasites. G. W. WHARTON, Duke University.

41. A Study of *Haemoproteus sacharovi* in Pigeons and Mourning Doves with Notes on *H. maccallumi* of Mourning Doves. G. ROBERT COATNEY, U. S. Public Health Service, AND EVALINE WEST, Peru State Teachers College.
42. Invasion of Young Red Blood Cells by Merozoites of Human and Avian *Plasmodium*. ROBERT HEGNER, Johns Hopkins University.
43. Exoerythrocytic Stages in the Development of *Plasmodium circumflexum*, and a Comparison of These Stages with *Toxoplasma*. REGINALD D. MANWELL AND FREDERICK GOLDSTEIN, Syracuse University.
44. Two Types of *Toxoplasma*-like Bodies in Canaries. FRUMA WOLFSON, Johns Hopkins University.
45. A Staining Technique for Demonstrating Avian Malaria Parasites in Tissue Sections. REDGINALD HEWITT, Johns Hopkins University.
46. Intestinal Helminths Found in Boys Recently Arrived in Washington, D. C., from Various Parts of the United States. ELOISE B. CRAM AND JOHN P. FOLAN, National Institute of Health.
47. Morphological, Physiological and Epidemiological Studies on *Eimeria bukidnonensis* Infection in a Group of Sixty Dairy Heifers. DONALD W. BAKER, N. Y. State Veterinary College, Cornell University.
48. *Lucilia* sp. Attacking Sheep at Beltsville, Maryland. PAUL D. HARWOOD AND A. C. JERSTAD, U. S. Bureau of Animal Industry.
49. Comparative Anatomy of Nemic Excretory and Reproductive Systems. M. B. CHITWOOD AND B. G. CHITWOOD, U. S. Bureau of Plant Industry, Babylon, N. Y.
50. Some Cercariae from Texas *Amnicola*. SEWELL H. HOPKINS, Agricultural and Mechanical College of Texas.
51. Embryology and Life Histories of Some Trematodes of the Genus *Plagioporus*. CHARLES G. DOBROVOLNY, University of Michigan.
52. *Mazocraes cepedianum*, a New Monogenetic Trematode from a Fresh-Water Fish. HARRY G. KIMPEL, University of Illinois. (Introduced by HARLEY J. VAN CLEAVE.)
62. The Life Cycle of *Stephanostomum tenuie* (Linton), Family Acanthocolpidae. (Also read) W. E. MARTIN, DePauw University.
65. Life History Studies on *Psilostomum ondatrae* Price and *Petasiger nitidus* Linton (Trematoda). (Also read) PAUL C. BEAVER, Lawrence College.
74. New Crustacean Parasites from the Atlantic Coast of North America. (Also read) A. S. PEARSE, Duke University.

FRIDAY MORNING SESSION, DECEMBER 30, 9:30 A. M... ROOM 209, MCGUIRE HALL, MEDICAL COLLEGE OF VIRGINIA.

*Read*

53. Parasite Studies on Ring-necked Pheasants, *Phasianus colchicus torquatus* (Gmelin) in Minnesota. (10 min) (Lantern) O. WILFORD OLSEN, University of Minnesota and Minnesota Conservation Department.
54. A Hawk Tapeworm which Produces a Proliferating Cysticercus in Mice. (5 min) LAWRENCE R. PENNER, University of Minnesota.
55. Variability in Hook Measurement in the Acanthocephala. (5 min) HARLEY JONES VAN CLEAVE, University of Illinois.
56. Larval Trematode Infection in Juveniles of *Physa parkeri* Currier. (15 min) (Charts) W. W. CORT, Johns Hopkins University, D. B. McMULLEN, University of Oklahoma, and LOUIS OLIVIER, New York University.
57. Host-parasite Relationship of Larval Trematodes in Oligochaete Worms. (10 min) (Lantern) CHARLES G. DOBROVOLNY, University of Michigan.
58. A New Heterophyid Cercaria from Texas. (8 min) (Opaque Projection) SEWELL H. HOPKINS, Agricultural and Mechanical College of Texas.

59. *Schistosomatium* from the Muskrat, *Ondatra zibethica*, in Minnesota and Michigan. (5 min) LAWRENCE R. PENNER, University of Minnesota.

60. A Strigeid of the Genus *Neodiplostomum* Which Develops in Laboratory Rats from a Diplostomulum Metacercaria in the Muscles of *Rana sphenocephala*. (5 min) LAWRENCE R. PENNER, University of Minnesota.

61. The Life Cycle of a Strigeid Belonging to the Diplostomidae. (12 min) (Lantern) LOUIS OLIVIER, New York University.

62. The Life Cycle of *Stephanostomum tenuis* (Linton), Family Acanthocolpidae. (10 min) (Lantern) (Also by demonstration) W. E. MARTIN, DePauw University.

63. Studies on the Pre-cercarial Development of *Stichorchis subtriquetrus* (Trematoda: Paramphistomidae). (15 min) (Lantern) HARRY J. BENNETT AND ARTHUR G. HUMES, Louisiana State University.

64. The Present Status of the Trematode Family Spirorchidae Stunkard. (10 min) (Lantern) ELON E. BYRD, University of Georgia.

65. Life History Studies on *Psilosostomum ondatrae* Price and *Petasiger nitidus* Linton (Trematoda). (15 min) (Lantern) (Also by demonstration) PAUL C. BEAVER, Lawrence College.

*By Title*

66. On the Life Cycle of a Tapeworm, *Diphyllobothrium* sp., from the Herring Gull, *Larus argentatus* Pont. LYELL J. THOMAS, University of Illinois.

67. Life History of the Cecal Fluke, *Postharmostomum gallinum*, of Poultry. JOSEPH E. ALICATA, University of Hawaii.

68. The Life Cycle of the Frog Bladder Fluke, *Gorgoderina attenuata* Staford, 1902 (Trematoda: Gorgoderidae). JOHN S. RANKIN, JR., Amherst College.

69. Observations on the Life History of *Spelotrema nicolli* n. sp. (Trematoda: Microphallidae), with the Description of a New Microphallid Cercaria. R. M. CABLE AND A. V. HUNNINEN, Purdue University, Oklahoma City University and the Marine Biological Laboratory.

70. The Life History of *Zygocotyle lunatum*. CHARLES H. WILLEY, New York University.

71. The Development of *Cercaria burti* Miller, 1923, in Leeches and Ducks. CHARLES H. WILLEY AND YALE RABINOWITZ, New York University.

72. Experimental Studies on *Posthodiplostomum minimum* (MacCallum, 1921) a Trematode from Herons. M. S. FERGUSON, Rockefeller Institute for Medical Research, Princeton, N. J. (Introduced by NORMAN R. STOLL.)

FRIDAY AFTERNOON SESSION, DECEMBER 30, 2:00 P. M.; ROOM 209, MCGUIRE HALL, MEDICAL COLLEGE OF VIRGINIA.

*Read*

73. Myxosporidia from Tide Pool Fishes of California. (7 min) ELMER R. NOBLE, Santa Barbara State College. (Introduced by HAROLD KIRBY, JR.)

74. New Crustacean Parasites from the Atlantic Coast of North America. (10 min) (Lantern) (Also by demonstration) A. S. PEARSE, Duke University.

75. Protopsiruriasis, a New Nematode Disease of Captive Monkeys. (10 min) (Lantern) A. O. FOSTER AND C. M. JOHNSON, Gorgas Memorial Laboratory, Panama.

76. Age Resistance of Rats against *Trypanosoma lewisi* and *Trypanosoma cruzi*. (15 min) (Lantern) J. T. CULBERTSON, M. H. KOLODNY AND C. J. DUCA, College of Physicians and Surgeons, Columbia University.

77. Effects of Number and Age of Worms on Development of Primary and Secondary *Hymenolepis diminuta* Infections in Rats (10 min) (Lantern) ASA C. CHANDLER, Rice Institute.

78. Specificity of Artificial Acquired Immunity to *Strongyloides ratti*. (10 min) A. J. SHELDON, School of Medicine, University of North Carolina.
79. Constitutionally Dissimilar Lines of *Strongyloides ratti*. (15 min) (Lantern) GEORGE L. GRAHAM, Rockefeller Institute for Medical Research, Princeton, N. J.
80. Studies on Dietary Deficiencies and Iron Salts in Experimental Canine Hookworm Infections. (15 min) (Lantern) G. F. OTTO AND J. W. LANDSBERG, Johns Hopkins University.
81. Three New Nematocides with a Consideration of Factors Governing Nematocidal Efficacy. (15 min) (Charts) B. G. CHITWOOD AND M. B. CHITWOOD, U. S. Bureau of Plant Industry, Babylon, N. Y.
82. Critical Tests with Iso-amyl-ortho-cresol for the Removal of Worms from the Dog. (15 min) A. C. JERSTAD, U. S. Bureau of Animal Industry.

*By Title*

83. The Effect of Dosage and Interval after Infection on Passive Immunity to the Nematode, *Nippostrongylus muris*. MERRITT P. SARLES AND WILLIAM H. TALIAFERRO, University of Chicago.
84. Rapid Loss of *Trichinella* Larvae Fed to Immune Rats and Its Bearing on the Mechanism of Immunity. O. R. MCCOY, University of Rochester.
85. A Note on the Cultivation of *Taenia taeniaeformis* Larvae in vitro. JAMES H. WILMOTH, New York University.
86. Development of the Microfilaria of *Dirofilaria scapiceps* (Leidy, 1886) in Mosquitoes of Minnesota. PAUL R. HIGHBY, University of Minnesota.

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## ABSTRACTS

### 1. *Balantidiasis*. MARTIN D. YOUNG, U. S. Public Health Service.

Nine infections of man with *Balantidium coli* are reported. Seven of these cases are described from South Carolina. Two cases were found in Tennessee by Dr. H. E. Meleney. Thirty-two cases from 16 states have been reported previously in the United States. In the cases from South Carolina, cysts were seen infrequently in five of the seven infections. It seems that these infections resulted from contamination with other human cases rather than from hogs, which are generally supposed to be the source of infection. Attention is called to the fact that infections may be more prevalent than have been reported.

### 2. *The Results of a Six-year Protozoological Survey of Food Handlers in a Collegiate Institution*. D. H. WENRICH AND JOHN H. ARNETT, University of Pennsylvania.

Examinations of food handlers for intestinal protozoa were conducted at a professional college in Philadelphia for 6 years up to 1937-38. During the first 3 years, the more permanent employees were examined; during the second 3 years, student helpers were also examined. Percentage incidences for the total of 291 examinations, some persons having been examined in successive years, were: *Blastocystis*, 71.1; some protozoa, 54.9; *Endolimax*, 34.4; *Endamoeba coli*, 22.6; *Giardia*, 11.7; *Endamoeba histolytica*, 6.5; *Dientamoeba*, 4.1; *Chilomastix*, 3.1; *Iodamoeba*, 1.7; *Enteromonas*, 0.7. For 143 examinations of the more permanent employees the percentage incidences were: *Blastocystis*, 74.1; some protozoa, 65.7; *Endolimax*, 44.7; *Endamoeba coli*, 25.9; *Giardia*, 14.7; *Endamoeba histolytica*, 4.2; *Dientamoeba*, 6.3; *Chilomastix*, 6.3; *Iodamoeba*, 2.8; and *Enteromonas*, 1.4. For 148 examinations of student helpers the percentage incidences were: *Blastocystis*, 68.9; some protozoa, 43.9; *Endolimax*, 25.0; *Endamoeba coli*, 19.6; *Giardia*, 8.8; *Endamoeba histolytica*, 8.8; *Dientamoeba* 2.0; *Iodamoeba*, 0.7; no *Chilomastix* or *Enteromonas*. All carriers of *E. histolytica* were treated as soon as detected and none of these showed infection with this species when examined in succeeding years.

### 3. *Comparative Efficiency of Various Technics for the Discovery of Protozoa and Helminths in Feces*. E. C. FAUST, W. SAWITZ, J. TOBIE, V. ODOM, C. PERES AND D. LINCICOME, The National Institute of Health and Department of Tropical Medicine, Tulane University.

Two hundred and seventy-one stool specimens from children's asylums and clinic patients were utilized in a comparative qualitative study. The methods compared were: the direct, iodine-stained fecal film, and after straining feces through cheesecloth and wire gauze; the hematoxylin-stained film direct and from a serum suspension of feces; centrifugation; flotation with a 33 per cent  $ZnSO_4$  solution, by touch, loop and superimposed coverslip removal of the surface film; hematoxylin-stained surface film levitated by  $ZnSO_4$  and removed by loop; and brine flotation with superimposed coverslip. No essential difference was found between films prepared directly from stools or after straining through cheesecloth or wire gauze; nor between touch, loop or superimposed coverslip removal of the surface film after levitation by  $ZnSO_4$ . The iodine-stained film technic discovered 45 per cent of the total protozoan infections detected by all technics employed, the hematoxylin film 60, centrifugation 46,  $ZnSO_4$  flotation 75. Percentage figures for detection of *E. histolytica* alone were 33, 39, 38 and 75 respectively.

### 4. *Hematoxylin Staining of Protozoan Cysts Obtained from Feces by Zinc Sulphate Levitation*. HANNAH S. NICKEL, Mississippi State Hygienic Laboratory, The National Institute of Health and Tulane University.

Once a suitable technic had been developed whereby protozoan cysts in human feces could be concentrated in a diagnosable state by zinc sulphate levitation (Faust *et al.*, 1938), it was desirable to learn if permanent hematoxylin-stained films of these concentrates could be prepared. The desired results were obtained as follows: (1) the levitated material was transferred to a clean slide with a platinum loop; (2) an equal amount of fresh blood-serum was mixed with the concentrate; (3) the wet film was fixed face downwards in Schaudinn's solution; (4) after washing in water and iodine solution, the film was mordanted for 20 minutes in one per cent ferric alum, stained for 20 minutes in iron-alum hematoxylin and then destained in one per cent ferric alum, washed, dehydrated, cleared and mounted in balsam. Most satisfactory results have been obtained from freshly passed feces. Thus far this technic has not been sufficiently perfected to recommend it for routine examinations in the clinical laboratory, since it requires considerable technical detail and great care. However, it provides a permanently stained film of concentrated cysts relatively free of fecal debris.

5. *The Presence of Pinworm (Enterobius vermicularis) Ova in Household Dust.* M. O. NOLAN AND LUCY REARDON, National Institute of Health, U. S. Public Health Service.

Dust from households, having members known to be infected with pinworms, was examined for ova of *Enterobius vermicularis*. Ova were found in the dust collected from the living room, dining room, kitchen, bath room, and bedrooms, at different levels, including the floor, baseboard, top molding of windows and doors, shelves and light fixtures, and from the tub, sink, and various pieces of furniture. In some instances, the viability of the ova was tested by hatching the active embryo in artificial digestive juice. In this medium, active larvae were hatched from ova collected from the floor and from the top molding of a door, as representing low and high levels of a room. It is pointed out that ova from high levels were beyond doubt air-borne, whereas those from low levels may have been air-borne, but not necessarily so. The finding of pinworm ova in household dust in its bearing on familial infection with pinworms is discussed. The ova of oxyurids found in household pests, such as mice, rats and cockroaches, were examined to preclude the possibility of their being confused with the ova of the human pinworm, *Enterobius vermicularis*.

6. *Comparative Efficiency of the NIH Anal Swab Examination and Stool Examination by Brine and Zinc Sulphate Flotation for Enterobius Infection.* W. SAWITZ, V. ODOM AND D. LINCICOME, The National Institute of Health and Department of Tropical Medicine, Tulane University.

One hundred and thirty-six children of an asylum were examined for *Enterobius vermicularis* infection by means of NIH anal swabs, and by the brine and zinc sulphate flotation of eggs in their stools. While brine flotation detected 14 per cent of the positives and zinc sulphate flotation 18 per cent, 73 per cent were found by the first swab examination. Consecutive anal swabs detected additional infections, 7 swabs giving a total of over 99 per cent of the total infections detected.

7. *The Treatment of Oxyuriasis with an Improved Type of Enteric-coated Tablet.* WILLARD H. WRIGHT AND FREDERICK J. BRADY, National Institute of Health, U. S. Public Health Service.

We have reported previously (1938, Proc. Helm. Soc. Washington 5: 5-7) that 112, or 91.8 per cent, of 122 cases of oxyuriasis treated with gentian violet were negative for pinworm ova on 7 consecutive post-treatment swabs taken 10 to 21 days after the end of the treatment. As a further safeguard on the validity of these results, on 19 additional cases we took 7 consecutive daily post-treatment swabs beginning with the 42nd day following the end of the treatment. Of these 19 cases, 11 or 57.9 per cent, were negative. This procedure involves the risk of

detecting also cases of reinfection, provided that *Enterobius vermicularis* requires 48 days or less for development. Some of the 8 positive cases in this group are known to have been exposed to reinfection. Through the kindness of Dr. John F. Kessel, we learned about a new type of enteric-coating. Gentian violet tablets with the new type of coating have been used in the treatment of about 50 cases of oxyuriasis, of which 31 have been carried to completion. Of these 31 persons, 25, or 80.7 per cent, were negative for pinworm ova on 7 consecutive daily post-treatment swabs beginning with the 42nd day following treatment. It is apparent that tablets with the new type of coating are superior to those used previously. Reactions encountered in patients treated with the new type of tablet have, in general, been less severe than those noted with the other type of tablet. The dosage and period of treatment are the same.

8. *Bufo marinus as a Vector of Helminth Ova in Puerto Rico.* W. A. HOFFMAN AND J. L. JANER, School of Tropical Medicine, San Juan, P. R.

The Surinam toad, *Bufo marinus*, imported into Puerto Rico about 18 years ago to combat insect pests, has increased tremendously. Many toads collected from various localities were found to be passing viable ova of *Ascaris lumbricoides*, *Trichuris trichiura* and *Schistosoma mansoni*. Ingestion of ova occurs when toads feed on insects developing in human excreta.

9. *Preliminary Report on the Incidence of Trichinosis in Alabama.* J. HENRY WALKER AND C. G. BRECKENRIDGE, University of Alabama.

Samples of the diaphragm, intercostal, rectus abdominis and pectoral muscles from 100 unselected routine autopsies at Birmingham and Tuscaloosa, Alabama, were examined. Thirty-three of the 100 were infected with larvae of *Trichinella spiralis*. The compression method of examination was used in the last 80 cases. All cases were examined by the digestion-Baermann technic. Quantitative counts of larvae were increased by stirring the muscle digest fluids in the funnels and by later sedimentation of muscle digest fluids in straight-walled jars. In the 33 positive cases the following numbers of positive muscles were found: 25 diaphragms, 20 intercostals, 21 recti, 20 pectorals. Of the 86 positive muscles, 6 were positive by the compression method alone, 52 by the digestion-Baermann technic alone and 28 by both methods. In 14 positive cases where larvae were detected in each of the four muscles the average numbers of larvae per 10 grams of muscle were 51.9 in the diaphragm, 7.1 in the intercostal, 5.2 in the rectus abdominis, and 4.4 in the pectoral. In 12 of these 14 positive cases where the diaphragm contained more than 1.0 larva per 10 grams as found by the digestion technic all four muscles were infected. There were no very heavily infected cases. Persons from the second through the ninth decades were represented in the series. Most of the 100 cases examined represented whites and negroes of low economic status. The completed survey will include an examination of 300 autopsy cases.

10. *Studies on the Protective Power of Serum from Dogs Actively Immunized Against Ancylostoma caninum.* G. F. OTTO, Johns Hopkins University.

(1) Four litter-mate puppies were given 5,200 hookworm larvae percutaneously. Two of these dogs were also given serum from an actively immunized dog. Both puppies receiving the serum escaped any serious effects of the pulmonary migration of the larvae, but one of them began to show the effects of the blood-sucking activity of the adult worms 18 days and died 25 days after infection; 1419 worms were recovered. The other dog receiving serum showed no physical signs of the infection, was killed on the 25th day and had 376 worms. The two control dogs became critically ill with verminous pneumonia at 9 days and died 13 and 20 days after infection; 2612 and 1348 worms were recovered. (2) In a second set of experiments infective hookworm larvae were incubated 1 to 3½ hours in (a) serum from an actively immunized dog, (b) in serum from previously uninfected puppies, (c) in physiological saline. Five hundred larvae

each from group (a) were injected subcutaneously (together with the serum) into five puppies in two litters; 500 from group (b) and (c) were similarly injected into four puppies for each group in the same two litters. All 13 puppies were killed 16 and 17 days after infection. Less than one-third as many worms were recovered from the five puppies receiving larvae treated with immune serum as were recovered from the other eight puppies.

11. *Schistosomiasis in Mountain Valleys of Venezuela.* J. ALLEN SCOTT, Ministry of Health, Venezuelan Government.

A program for the study of schistosomiasis in Venezuela has for the first year been confined to mountain valleys at an altitude of about 3000 feet and near Caracas. The snails transmitting the infection are found exclusively in the canals used for irrigation of the rather flat bottoms of these valleys. Throughout the valley of the River Valle about 70 per cent of the peasants were found infected. In other valleys the prevalence varied from 30 to 75 per cent in different places. Although egg counts of this species are still of unproved value, it is interesting to note that the average egg output is apparently about equal to that in the most heavily infected parts of Egypt. The crux of possible control seems to lie in the provision of domestic water supplies apart from irrigation canals, for then the larger canals could be covered near houses, and the smaller ones drained by modifications of methods used for the control of malaria. Whether these conditions are typical of all infected areas in Venezuela can only be determined by further study.

12. *Schistosomiasis Due to Schistosoma mansoni and Its Transmission in Ethiopia.* ARNALDO GIOVANNOLA, Italian Institute of Public Health, Rome.

The author has observed at Harar (Ethiopia) two cases of intestinal schistosomiasis due to *Schistosoma mansoni*, which are possibly the first two cases of this disease reported in Ethiopia. With one of the patient's feces containing eggs of *Schistosoma* it has been possible to infect three snails collected in the country, obtaining cercariae of *S. mansoni* 31 days after contact. The snails have been classified as *Planorbis adowensis* Bourguignat, 1879.

13. *Notes on the Incidence of Human Intestinal Helminths in the State of Cojedes, Venezuela, South America.* WILLIAM HUGH HEADLEE, Purdue University.

In conjunction with the National Educational Program of Venezuela, 499 persons living in the State of Cojedes were examined for intestinal helminths, being selected at random from seven villages and towns of the state. One fecal specimen from each individual was examined by the direct smear method. The infections noted and the percentages of incidence were as follows: *Ascaris lumbricoides*, 60.5, *Trichuris trichiura*, 58.9, and hookworm (probably *Necator americanus*), 40.1. Four hundred and forty-six, or 89.4 per cent, were infected with one or more of the three species. Among 268 individuals of school age (6-16 years), including 54 per cent of the total persons examined, the percentages of incidence were as follows: *Ascaris*, 64.6, *Trichuris*, 64.9, and hookworm, 50.7. Two hundred and fifty, or 93.3 per cent were infected with one or more species of these helminths. Hill (1929) reported the results of examinations of 143 persons from one of these population groups, Tinaquillo, both the Willis and Caldwell methods being used to make examinations. He noted the following infections and percentages of incidence: *Ascaris*, 67, *Trichuris*, 60, and hookworms, 84, while 93 per cent of this group had an infection with one or more species of the helminths. In the present survey of Tinaquillo 63 persons were examined, the following incidence percentages being noted: *Ascaris*, 70, *Trichuris*, 78, and hookworm, 27, with 89 per cent of the individuals having an infection with one or more of the three species of helminths.

14. *The Possible Rôle of Birds in the Epidemiology of Sylvatic Plague.* WILLIAM L. JELLISON, Rocky Mountain Laboratory, U. S. Public Health Service, Hamilton, Montana.

Field and laboratory studies made in the plague area of southwestern Montana during 1936 and 1937 indicate that predatory birds are potential agents in the dissemination of plague. Birds of prey were found to carry rodent fleas either on captured rodents or as accidental parasites. A few rodent fleas were found on fledglings, adults and in the nests of great horned owls and American long-eared owls. The occupied underground nest of a burrowing owl yielded 107 live rodent fleas of 5 species that had obviously been carried to the nest on rodent carcasses. *Bacillus pestis* was repeatedly isolated from regurgitated casts of fledglings of the great horned owl, American long-eared owl and Swainson hawk following the ingestion of infected guinea pig tissue. Whether or not casts of birds are ever eaten by rodents is uncertain. In five instances *B. pestis* was recovered from ground squirrels, *Citellus richardsoni*, being eaten by birds. Two of the squirrels had been carried some distance. As ground squirrels are often cannibalistic they may become infected by eating refuse discarded at feeding perches or at nests remote from areas of established infection. Crows and magpies were seen feeding on infected rodents, but were not observed carrying rodent carcasses and only one rodent flea was found on those examined. These scavengers may actually inhibit the spread of plague by rapid destruction of rodents dead of the disease.

15. *The Tick, Ornithodoros hermsi, as Probable Vector of Relapsing Fever in Northern Idaho.* CORNELIUS B. PHILIP AND GORDON E. DAVIS, Rocky Mountain Laboratory, U. S. Public Health Service, Hamilton, Montana.

Temporary occupants of a summer cabin on the wooded slopes of Moscow Mountain, near Moscow, Idaho, reported being bitten by ticks during the summer of 1931. Specimens taken by us in this cabin in 1932, using animal bait, were identified as *Ornithodoros hermsi*, the first report of this species north of California. In August, 1938, four of six persons sleeping at the cabin developed illnesses suggestive of relapsing fever; spirochaetes were found in blood smears taken during the 3rd relapse of one patient, and during the 4th and 5th relapses of another. Also a strain was established in white rats from blood of the latter patient. This is the first confirmed recognition of relapsing fever in Idaho. Two other persons occupying this cabin have had undiagnosed illnesses suggestive of the disease, the owner in 1930 and a visitor in 1936. In 1938, a 3rd suggestive case involved a permanent resident in another cabin about 10 miles distant. Tests in white rats of four adult and four immature *O. hermsi* collected in the cabin in 1932, and of three nymphs taken in 1938, resulted negatively. Several attempts to recover ticks from nearby dwellings and from local rodents, their burrows and nests, or to demonstrate the infection in rodent brains have been without success.

16. *Blood Alterations in Typhlohepatitis of Turkeys, with Notes on the Disease.* E. P. JOHNSON AND C. J. LANGE, Virginia Agricultural Experiment Station, Blacksburg, Va. (Introduced by BRUCE D. REYNOLDS.)

*Histomonas meleagrididis* in cecal discharges of "carrier chicks," when introduced into the rectum of turkeys held in a suspended position, is extremely pathogenic as compared to other forms of this protozoan and to other channels of administration. The average differential leucocyte count of normal turkeys maintained under the conditions of this experiment is as follows: heterophiles 43.4; eosinophiles 0.9; basophiles 3.2; lymphocytes 50.6; monocytes 1.9 per cent. Experimental blackhead disease of turkeys results in a marked disturbance in the blood picture which is characterized by: (1) a marked heterophilia of from 8 to 21 per cent increase within 24 hours of receiving the infective material; (2) a persistence of the heterophilia until death; (3) an irregular monocytosis in some

cases, especially in those receiving subcutaneous implantations of infective material; (4) myelocytosis and anemia usually preceding the fatal termination. The sudden marked heterophilia following introduction of infective material indicates that the histomonads penetrate the cecal epithelium and establish themselves in some parenteral position where toxic material results within 24 hours. Natural cases of typhlohepatitis were found to have leucocyte disturbances very similar to those found in the experimentally infected birds. An extensive lesion in the proventriculus, due to the administration of *H. meleagridis* by way of the mouth, is described and illustrated. In no case could intravascular protozoan forms be demonstrated in the experimentally infected birds.

17. *Cultures of Pentatrichomonas sp. in vitro from the Livers of Chickens and Turkeys.* ENA A. ALLEN, U. S. Bureau of Animal Industry.

By using a modification of Boeck and Drbohlav's medium, nine tubes of pure cultures of *Pentatrichomonas* sp. were obtained from the liver of a turkey which had died two hours previously from some unknown cause. The cultures remained free from bacteria, and the protozoa survived for eight days, following two transplants to fresh media. A pure line of this strain of *Pentatrichomonas* grew indefinitely with a single species of *Staphylococcus*. Fresh mounts from the livers of two apparently normal chickens, previously infected experimentally with *Pentatrichomonas* species through the oral route, did not reveal any pentatrichomonads. However, *Pentatrichomonas* sp. was recovered in cultures from the livers of these birds. The ceca were heavily infected with pentatrichomonads, but the organisms were absent from the small intestines. It is probable that the protozoa reached the liver through the blood stream.

18. *Domestic Fowls as Carriers of the Poultry Gapeworm.* EVERETT E. WEHR, U. S. Bureau of Animal Industry.

In connection with the control of the gapeworm, *Syngamus trachea*, in poultry, it is important to know what birds are susceptible to infection and how long each type of host may remain a carrier of the parasite. Obviously, those birds which remain infected longest are potentially the most dangerous. To determine the duration of infection, various kinds of poultry were experimentally fed infective gapeworm material. The birds used in the experiment had been hatched in an incubator and reared under parasite-free conditions until infected. After infection, they were held under parasite-free conditions until the experiment was terminated. After the 14th day of infection, fecal examinations of each infected bird were made at bi-weekly or weekly intervals. Whenever the feces were found negative for gapeworm eggs for two successive examinations, the bird was destroyed and examined for gapeworms. Tracheal infections occurred in turkeys, chickens and guinea fowls, but not in pigeons and ducks. In the pigeon, immature larvae were found in the lungs in several cases. Chickens were found most susceptible to gapeworm infection when young, but turkeys and guinea fowls were susceptible at all ages. The data obtained show that chickens infected when very young may retain their infections for as long as 147 days, guinea fowls for 98 days and turkeys for 224 days.

19. *Goblet Cells and Age Resistance to Parasitism.* J. E. ACKERT AND S. A. EDGAR, Kansas State College.

In a study of possible factors in age resistance of chickens to the duodenal nematode *Ascaridia lineata* (Schneider), histological examinations were made of the epithelial lining of the duodenum of chickens from two to 320 days of age. The number of goblet cells per given area of duodenal epithelium increased gradually with the age of the chickens up to 124 days. Counts of goblet cells in areas of the same size gave averages as follows: two-day chicks, 2.9 goblet cells per area; five-day, 3.7; 12-day, 5.0; 26-day, 7.3; 40-day, 7.7; 58-day, 8.7; 71-day, 9.3; 124-day, 10.7; 131-day, 9.2; and 320-day chicks, an average of 9.0 goblet

cells per area. These increases in goblet cells in chickens up to 124 days of age correspond closely to the manifestations of age resistance to numbers of *A. lineata* in chickens. The numbers of goblet cells appear to reach their maximum in chickens at about three or four months of age. So also does the resistance of fowls to the numbers of *Ascaridia lineata* reach its maximum in chickens three to four months of age. That secretions of the goblet cells may be a factor in the elimination of *A. lineata* from the host is conceivable since it was observed repeatedly on opening the duodenum that mucus was much more abundant in the older chickens than in the younger ones. Apparently, this is the first record of increased numbers of goblet cells in older animals, and of their possible relation to age resistance to parasitism.

20. *Effects of the Tapeworm Raillietina cesticillus (Molin) on Growing Chickens.* J. E. ACKERT AND A. A. CASE, Kansas State College.

Two experiments have been carried out to determine effects of the fowl tapeworm *Raillietina cesticillus* (Molin) on growing chickens (White Leghorns). The cysticercoids used for infecting the chickens were grown in beetles (Coleoptera). In the first experiment, which was of 24 days' duration, 50 cysticercoids were given to each chicken, several cysticercoids a week. Studies were made of the comparative growth, hemoglobin percentage and the blood sugar content of 18 parasitized and 7 control chickens 3½ months of age. Results of the first experiment: average gain in weight, parasitized chickens, 102 gm, controls, 167 gm; hemoglobin, parasitized chickens, 63 per cent, controls, 62 per cent; blood sugar, parasitized chickens, 168.7 mg per 100 cc blood, controls, 190 mg. In the second experiment which was of eight weeks' duration, 80 cysticercoids were given to each chicken, ten per week. This test was made upon ten parasitized and four control chickens, all four months of age. Results of the second experiment: average gain in weight, parasitized chickens, 720 gm, controls, 967 gm; hemoglobin, parasitized chickens, 55 per cent, controls, 60 per cent; blood sugar, parasitized birds, 141.1 mg per 100 cc blood, controls, 176.6 mg. The results of the two experiments, in which the parasitized chickens had from 4 to 25 worms, indicate that infections of *Raillietina cesticillus* of three to eight weeks' duration tend to reduce the growth rate of chickens and the sugar content of the blood, and in eight weeks the hemoglobin percentage also. This appears to be the first record of lowered blood sugar content and hemoglobin percentage due to tapeworm infection.

21. *Susceptibility of Chickens to Reinfection with Raillietina cesticillus as Determined by the Presence of the Original Terminal Segment.* GEORGE W. LUTTERMOSER, U. S. Bureau of Animal Industry.

The original terminal segments of tapeworms have a characteristic structure which differentiates them from other proglottids of the strobila. These terminal segments are shed before or soon after the tapeworms become mature. The presence of the original terminal segment may, therefore, be used as a means for the detection of recently acquired infections and for recognizing superimposed infections. Fifty-three parasite-free Rhode Island Red chickens 14 to 28 days old were divided into three groups of 7, 22, and 24 birds, respectively. Each bird in groups I and II received from 100 to 350 cysticercoids of *Raillietina cesticillus*. Six to 12 weeks later, each chick of group II received an additional 50 cysticercoids, and each chick of group III received 50 cysticercoids for the first time. Ten days later, all the birds were autopsied. The birds in group III harbored an average of 7.6 worms bearing original terminal segments; this represented 45.6 per cent of the average total infection. In group II, an average of 5.4 worms per bird or 5.3 per cent of the average total infection bore original terminal segments. However, the average number of worms per bird (5.4) with original terminal segments found in group II represents 32.4 per cent of the expected number of worms, assuming that the chickens were nonresistant to

reinfection. The difference in numbers of worms bearing original terminal segments removed from groups II and III is not statistically significant. These results demonstrate that 6 to 12 weeks after receiving a relatively heavy infection of *R. cesticillus*, the young chickens apparently had acquired little or no resistance to reinfection with this tapeworm.

*22. A Method for Testing the Rôle of Host Activity in Coccidian Periodicity.*  
DONALD C. BOUGHTON, University of Georgia.

To locate the important periods of daily host activity governing parasite periodicity would appear to be a first step toward an elucidation of the underlying mechanism involved. Extended experimental changes in host schedule will not serve to single out particular periods, because they involve the entire sequence. The diurnal periodicity of oöcyst production (*Isospora in Passer d. domesticus*) has been found to be sensitive to certain 6-hour changes in host activity, the effects of which can be observed after the hosts have resumed their normal schedule. For example, when light and food are supplied to birds beginning 6 hours before daylight on the first day of observation, the oöcysts are released ahead of time on the second and third days, during which normal host activity is maintained; the oöcysts appear at the normal time on the fourth and succeeding days. A similar effect is noted when birds receive light but no food for the same 6-hour interval. These and other observations obtained by means of similar short experiments suggest that normal oöcyst periodicity bears a definite relation to the time of day at which the host undergoes a change from the resting to the active state. It is believed that this type of experiment is applicable to the testing of such drastic treatments as lack of food, temperature changes, drug administration, etc., which, if prolonged, would seriously affect the host.

*23. Dried Milk Products in the Ration and Mortality with Cecal Coccidiosis in Chicks.* ELERY R. BECKER AND P. H. WATERS, Iowa State College.

Previous experiments have demonstrated the unfavorable effect of dried buttermilk on the course of *Eimeria tenella* infection in White Leghorn chicks when it is fed in the "ordinary" type of ration. It is now clearly shown that both dried buttermilk and dried skimmilk in the ration occasion higher mortality than the absence of these materials, when a sardine meal dried at low temperatures is used as the source of animal protein. A start has been made in the direction of determining which of the constituents of dried milks are objectionable from the standpoint of coccidiosis control. Preliminary experiments have indicated that when part of the protein in dried buttermilk is replaced with crude casein, mortality is considerably less than when it is replaced with whey. Likewise, replacement with soy bean oil meal (expeller process) seems to have a mitigating effect on mortality. Replacing part of the protein in meat scraps with that of soy bean oil meal did not decrease the severity of the infection. It is significant of progress that weights of the chicks are being maintained along with the constructing of rations that make the course of the infection less severe.

*24. Observations on Eimeria bukidnonensis in New York State Cattle.*  
DONALD W. BAKER, Veterinary College, Cornell University.

The first reported observation of bovine infection with *E. bukidnonensis* in North America is described. This report presents new and confirming evidence of the specificity of *E. bukidnonensis*, and that it is a true bovine parasite. Measurements from a wide sampling in a group of 60 experimental heifers indicate the presence of two distinct size groupings. Results from routine monthly fecal examinations from the 60 animals show the gradual transmission of the infection from one animal in October, 1937, to the entire group by October, 1938. Investigation of the physiological characteristics of the exogenous phase of the life cycle establishes the sporulation period at room temperature in shallow culture at from 24 to 27 days in contrast to the short periods required for the other and

more commonly found species of bovine coccidia. The case record of an experimental infection in a susceptible calf shows the production of large numbers of oocysts on the 10th day following oral administration of 55 sporulated oocysts. Photomicrographs depicting the developmental changes occurring during the period of sporulation include illustrations of the double layered wall of the oocysts. Our findings support the theory of Henry of the occurrence of separable walls surrounding the oocyst.

*25. Fifteen Cases of Experimental Bovine Venereal Trichomoniasis.* CHAS. W. REES, U. S. Bureau of Animal Industry.

In 10 cases of the disease in females, in which transmission was effected either through coitus or through intravaginal inoculation of *Trichomonas foetus* in pure culture, the fecundity of the host has been tested with the following results: one heifer failed to have estrum; one pregnant heifer that was exposed during gestation, showed no ill effects; 5 heifers and 2 cows, the latter not having been previously exposed, developed breeding troubles; failure of early conception and disturbances of estrum were the outstanding effects; finally, after recovering from the infection, all animals conceived, 4 have already calved, 3 are due to calve. One heifer developed pyometra and for 18 months the vaginal discharges have contained *T. foetus*; 20 quarts of pus containing the parasites in pure culture have been aspirated from the uterus of this animal. In 4 cases, involving cows that had been exposed as heifers, the fecundity was also tested after reexposure by coitus with an infected bull. These cows became positive for *T. foetus*, but conceived without appreciable delay. A bull that contracted the disease from a heifer has been positive for *T. foetus* for 20 months. To all of 10 females served, this bull has transmitted the parasites, regardless of whether the females had been previously infected. The incubation period in these cases was about 12 days. Two control heifers conceived at the first service by a non-infected bull and bore calves after a normal gestation.

*26. Sterile Culture of the Free-living and Parasitic Larval Stages of Haemonchus contortus.* R. W. GLASER AND NORMAN R. STOLL, Rockefeller Institute for Medical Research, Princeton, N. J.

Two aspects of the culture of *Haemonchus contortus* are described. The one deals with the free-living stages grown steriley; the other with the early parasitic stages similarly grown. A medium was devised in which the eggs, freed of adhering bacteria, hatched and the larvae developed to the infective stage. These larvae were morphologically and otherwise normal, except that they were slightly smaller than fecal-grown specimens although the size ranges overlapped. Infection tests proved that *Haemonchus* larvae grown under conditions of sterility were normally infective for, and produced normal adults in, a susceptible lamb. To obtain the development of the parasitic stages, it became necessary to use those that had reached the end of their free-living stage in sheep feces. These were subjected to a prolonged washing procedure. To expedite the sterilization and also to unsheathe the infective filariform larvae, a dilution of Labarraque's solution was used. After unsheathing and sterilizing, the larvae were inoculated into a special medium different from that used for the free-living stages. In this medium, under incubation, the nematodes developed through the fourth larval (second parasitic) stage with growth and differentiation, reaching a size sometimes exceeding 3 mm in length. Even in smaller sizes males and females were readily differentiated. They were about five times the length of the unsheathed larvae with which the culture tubes had been inoculated. As yet we have not seen adult worms in our cultures.

*27. The Efficacy of Phenothiazine for the Removal of Ascarids and Nodular Worms from Swine.* PAUL D. HARWOOD, A. C. JERSTAD AND LEONARD E. SWANSON, U. S. Bureau of Animal Industry.

Because of the difficulty in administering anthelmintics to swine, there is a demand for a worm remedy that can be given in the feed. Existing data suggest that phenothiazine may be effective for the removal of nodular worms and ascarids from swine when administered in such a manner. In preliminary experiments phenothiazine was given to 5 pigs in varying doses and by various methods of administration. The results obtained indicated an efficacy against nodular worms of 25, 93, 100, 93, and 100 per cent, respectively, and against ascarids of 100, 0, 37, 50, and 100 per cent, respectively. For both types of worms 100 per cent efficacy was obtained when the dose rate was 1 gram of the drug per kilo of live weight, and when the dose was administered in 4 times its weight of feed. This method was adopted for tests on two additional pigs. From the first pig, 84 ascarids (94 per cent of the total present) and 61 nodular worms (98 per cent) were removed. From the second pig, 1,136 nodular worms (98 per cent) were removed; no ascarids were present. Phenothiazine showed little promise as an anthelmintic for the removal of stomach worms, thornyheads, hookworms, and whipworms. Host reactions, which may have been caused by the drug, were red-stained urine and constipation in some of the animals treated.

28. *New Intermediate Hosts of the Fowl Tapeworm* Raillietina cesticillus (Molin). A. A. CASE AND J. E. ACKERT, Kansas State College.

Further studies on the transmission of fowl tapeworms in the vicinity of Manhattan, Kansas, have yielded four new findings. Beetles (Coleoptera) collected at some distance from any poultry yard were kept in the laboratory for a few days and then given one or two gravid proglottids of the fowl tapeworm, *Raillietina cesticillus* (Molin). The beetles were then placed in culture jars containing soil and kept moist by the addition of water to filter paper. They were fed on meat-scrap for a period of about three weeks when they were killed and examined for cysticercoids. The latter were then fed to chickens to test their viability. By this method the following Coleoptera have been found as new intermediate hosts for *R. cesticillus*: *Harpalus herbivagus* Say, *Podabrus modestus* Say, *Tenebroides mauritanicus* (L.) and *Anaferonia* sp. (near *substriata* Lec.). *Triplectrus rusticus* Say is reported for the first time from Kansas as an intermediate host of this tapeworm.

29. *Barium Antimonyl Tartrate as a Remedy for the Removal of Gapeworms from Chickens*. EVERETT E. WEHR, PAUL D. HARWOOD AND JACOB M. SCHAFER, U. S. Bureau of Animal Industry.

A large number of finely powdered drugs were administered to chickens by inhalation to determine their efficacy in removing gapeworms. Of the substances tested, barium antimonyl tartrate proved to be the least toxic and the most effective for the purpose indicated. For treatment, 3 to 5 chicks were placed in a glass battery jar, 9 inches wide and 12 inches deep. A small amount of the powdered drug to be tested was sprinkled on the head of each bird and the remainder emptied into the jar; the jar was then covered with a cloth or paper towel. The loose powder in the jar was then blown forcibly into the air by means of a large rubber bulb fitted to a glass tube inserted at one side of the cover. The birds were usually exposed to the dust for 10 minutes, and during that time the powder that had settled to the bottom of the jar was redispersed two to four times. After treatment, the birds were returned to clean quarters and kept there four to six days; the birds were then killed and the tracheae examined for gapeworms. In the ten experiments with barium antimonyl tartrate, 143 infected birds were treated and only four pairs of live worms were recovered at autopsy, whereas from 48 control birds, 94 pairs of worms were recovered. The average indicated efficacy of the treatment in the ten tests is slightly more than 98 per cent.

30. *Hemorrhage as the Cause of the Fatal Anemia Associated with Stomach Worm Infection in Sheep*. JOHN S. ANDREWS, U. S. Bureau of Animal Industry.

Two parasite-free lambs, weighing approximately 25 pounds each, were experimentally infected with 100,000 and 120,000 infective larvae of *Haemonchus contortus*, respectively. Prior to administration of larvae the blood of these lambs was normal, containing 13.04 and 14.78 million red cells per cu mm, 12 gm hemoglobin per 100 cc, and having a cell-volume percentage of 33 and 37, respectively. The feces of these lambs were treated daily with benzidine and hydrogen peroxide in order to detect occult blood. Ten and 11 days, respectively, after infection, blood was first found in considerable quantity in the feces. All feces passed by the lambs were then collected at the end of each 24-hour period, and the quantity of blood estimated by means of a modification of van Eck's method (1924). The lambs lived 12 and 14 days, respectively, after the first appearance of blood in the feces. At death, the blood of these lambs was found to contain 2.2 and 2.02 million red cells per cu mm, 2.25 and 1.6 gm hemoglobin per 100 cc, and to have a cell-volume percentage of 9 and 7, respectively. Post-mortem examination showed that both lambs were heavily infected with *H. contortus*. It was estimated that the lambs lost approximately 1,500 and 2,200 cc of blood, respectively, during the 12 and 14 days of continuous hemorrhage. The same degree of anemia was produced in a third lamb by removing a like percentage of the total blood volume during approximately the same length of time by daily bleeding from the jugular vein.

31. *A Study of the Periodicity and Synchronicity of the Pigeon Strain of Plasmodium relictum*. G. ROBERT COATNEY, U. S. Public Health Service.

This strain of *P. relictum* was isolated in 1937 and has been maintained in the pigeon since that time. Fourteen birds have been used in this study. The strain shows a decided periodicity with an asexual cycle of from 24 to 30 hours. The infections are relatively synchronous.

*Cross Immunity Reactions in Avian Plasmodia*. FRUMA WOLFSON, Johns Hopkins University.

Four strains belonging to two species of *Plasmodium* were inoculated into 38 canaries. In three groups of experiments one strain was injected into canaries previously infected with another strain. Blood films were prepared daily from these birds, the number of parasites counted, and the results combined into tables. (1) *Plasmodium (praecox) relictum*, matinal strain vs. *P. cathemerium*, the Hartman strain from a sparrow and the Wolfson strain from a wood thrush. Five canaries were infected with *P. relictum* during a period of from 18 days to over two years, and then superinfected with the Hartman strain of *P. cathemerium*. Four canaries exhibited a typical *P. cathemerium* infection. One bird showed no parasites after the second inoculation, but a clean canary inoculated with its blood had a mixed infection. A second series of nine canaries were first inoculated with the Hartman strain of *P. cathemerium* and reinoculated from 44 to 222 days later with *P. relictum*. None showed a normal *P. relictum* infection. These and other experiments indicated that *P. cathemerium* produced a rather high degree of immunity to a superinfection with *P. relictum*, but the latter strain produced little or no immunity to superinfection with *P. cathemerium*. (2) Further experiments supported the conclusion previously reported (Wolfson, 1937) that the matinal strain of *P. relictum* produces a very slight immunity to superinfection with the strains belonging to either *P. relictum* or *P. cathemerium*. But any of the three strains used, produce, if not a complete, at least a partial immunity to reinfection with the matinal strain of *P. relictum*. (3) The Hartman and Wolfson strains of *P. cathemerium* resemble each other in the synchronicity of sporulation, but differ in the time of sporulation and in the fact that the Hartman strain does not usually kill the canaries, nor exhibit *Toxoplasma*-like bodies in tissues, whereas the Wolfson strain kills the host and exhibits *Toxoplasma*-like bodies. Six canaries inoculated with the Hartman strain were inoculated with the Wolfson strain 161-323 days later. Three controls and one

experimental bird died as a result of the infection. Five of the experimental birds showed no parasites. Thus, the Hartman strain of *P. cathemerium* produced in five of six cases a very strong immunity to reinfection with the Wolfson strain of the same species.

33. *Studies on Immunity in Avian Malaria, with Special Reference to Plasmodium circumflexum.* REGINALD D. MANWELL AND FREDERICK GOLDSTEIN, Syracuse University.

The problem has been attacked from three angles: (1) the behavior of different strains of the same species (mainly *circumflexum*) when crossed on one another, (2) the behavior of different species when crossed on one another, and (3) the possibility of artificially producing an acquired immunity by the injection of immune serum. In general, it has been demonstrated that the presence of a chronic infection of any of the six strains of *Plasmodium circumflexum* used (isolated originally from five species of hosts and three widely separated geographical regions) protected against any of the other five, but there were considerable differences in the degree of immunity conferred, and it was also observed that there was a good deal of difference in the behavior of different cases. One strain (originally from a red-winged blackbird), in particular, showed an ability to produce rather acute infections in birds carrying chronic infections of other strains. When one species was crossed on a chronic infection of another, the existence of an immunity (not always reciprocal) was frequently observed. Although the species protecting against one another were often similar morphologically, and hence presumably related, there were numerous exceptions, e.g., *Plasmodium rouxi* gives a strong protection against later infection with *circumflexum* (even when different strains of the latter species are used), but there is no reason to think there is any close relationship between these species. And the reverse cross shows that the immunity is not reciprocal. Efforts to confer passive immunity by the injection of large doses of serum from chronic cases were successful, and served to prevent completely (or much modify the severity of an infection when one resulted) the occurrence of infection after the injection of blood containing relatively large numbers of parasites. In some cases the immune serum was administered in daily doses for some days prior to parasite inoculation, and in others for some days subsequently. The former method was successful in completely preventing infection.

34. *Cellular Reactions to Malaria in the Skin of Normal and Immune Canaries and Monkeys.* WILLIAM H. TALIAFERRO AND WILLIAM BLOOM, University of Chicago.

The skins of 43 non-immune and 43 immune (latently infected with *Plasmodium cathemerium*) canaries were injected with 0.01 cc of trypan blue on one leg and with the same amount of homologous malarial blood cells on the other leg. The 86 pieces of skin were thereafter removed at closely spaced intervals beginning with 5 minutes and extending over several weeks. Similar experiments with *P. brasiliense* in normal and immune (latently infected with *P. brasiliense*) spider and *Cebus* monkeys were performed except that normal blood cells were injected, instead of trypan blue, and because fewer animals were available, several of the monkeys had 10 to 16 injections on various parts of their body. No differences could be found either in the intensity of inflammation or in the phagocytic activity of the macrophages in the immune, as compared to the non-immune animals. These findings, together with the inability to demonstrate humoral protective antibodies in the immune animals (1929, W. H. and L. G. Taliaferro, J. Prev. Med. 3: 209; and 1934, Am. J. Hyg. 20: 60), indicate that the opsonic antibodies, postulated to account for the greatly accelerated specific phagocytic activity of the macrophages of the spleen, liver and bone marrow in immune animals (1931, Cannon and W. H. Taliaferro, J. Prev. Med. 5: 37; and 1936, W. H. Taliaferro and Cannon, J. Infect. Dis. 59: 72), occur in too low concen-

trations in the skin of immune animals to accelerate the action of the macrophages in the skin.

35. *Comparative Study of the Morphology and Life History of Two Species of Frog Filaria.* O. R. CAUSEY, Johns Hopkins University.

Two species of filaria were found in frogs from the southern United States. *Foleyella americana* was found in *Rana pipiens*, and an undescribed species was found in *Rana aesopus*. The adult worms and the microfilariae of these two species have been studied, and the development of the larvae has been traced from the microfilaria to the infective stage in two species of mosquitoes.

36. *The Relation of Shade to Anopheles quadrimaculatus Breeding.* E. HAROLD HINMAN AND HERBERT S. HURLBUT, Tennessee Valley Authority, Wilson Dam, Ala.

In various parts of the world certain species of anophelines have been controlled through the introduction of shade. In the reservoirs of the Tennessee Valley Authority many marginal acres of timber are cut in reservoir preparation for malaria control. To determine if shade may be employed in the limitation of *Anopheles quadrimaculatus* breeding this investigation has been undertaken. Certain experimental areas, subsequently inundated, were left uncleared to observe if breeding occurred. The planting of aquatic trees in marginal areas has been introduced. Studies of the amount of breeding in naturally shaded areas during the past two summers have been carried on. Artificial shade has been created in a natural breeding area by an overhanging tarpaulin. These studies, together with the observed ability of *Anopheles quadrimaculatus* to develop in the complete absence of light, have led to the conclusion that shade has only an indirect influence upon the breeding of this species. When of sufficient density, shade may diminish, but not entirely inhibit, breeding.

37. *Acquired Immunity to Ticks.* WILLIAM TRAGER, Rockefeller Institute for Medical Research, Princeton, N. J.

One infestation of guinea pigs or rabbits with larvae of the American dog tick, *Dermacentor variabilis*, induces an acquired immunity which effectively prevents subsequent batches of larvae from engorging. The immunity develops fully within two weeks after the start of the first infestation and lasts at least three months. There is a cross-immunity between larvae of *D. variabilis* and *D. andersoni* on guinea pigs, and between larvae of *D. variabilis* and *Haemaphysalis leporis-palustris* on rabbits. Deer mice become relatively resistant to larvae of *D. variabilis* after 2 or 3 infestations. The repeated infestation of guinea pigs with nymphs or adults of *D. variabilis* results in a marked reduction in the amount of blood taken by ticks of the later batches. The immunity of guinea pigs to larvae of *D. variabilis* can be produced by the intracutaneous inoculation of an extract of larval ticks or of the salivary glands of adult ticks. It can be passively transferred by the intraperitoneal inoculation of serum from guinea pigs hyperimmunized by repeated infestations with nymphs. In the ears of non-immune guinea pigs, four days after attachment of a larva of *D. variabilis*, there is little cellular reaction at the site of attachment. In immune animals, there is present by the fourth day an intense inflammatory reaction. A mass of leucocytes surrounds the mouthparts of the tick and the epithelium has thickened and begun to grow beneath the leucocytic mass, thus walling off the tick from its source of supply of blood.

38. *Some Problems in Medical and Veterinary Entomology.* F. C. BISHOPP, U. S. Bureau of Entomology and Plant Quarantine.

President's address.

39. *The Localization of Glycogen in Macracanthorhynchus hirudinaceus.* THEODOR VON BRAND, Barat College of the Sacred Heart, Lake Forest, Ill.

The localization of glycogen has been studied with morphological methods by several workers in Nematoda, Trematoda and Cestoda, but so far nothing is known about its distribution in the body of Acanthocephala. A similar study has, therefore, been made in *Macracanthorhynchus hirudinaceus*, using Best's glycogen stain. Relatively large amounts of glycogen were found in the hypodermis and in its canal system. This demonstrates that the thick skin of these parasites is not only an organ of resorption, but also of deposition of reserve substances, and even perhaps of synthesis. Much glycogen was also found in the protoplasmic parts of the muscles, especially those connected with the proboscis and the reproductive organs. The floating ovaries were found to contain little glycogen, the early developmental stages were glycogen-free, but a noticeable amount of polysaccharide appeared in the later stages, concentrating in the region of the "Embryonalkern." In the males, the testes were glycogen-free, but some glycogen was found in the cement glands. Small amounts of glycogen were found in the cells of the proboscis ganglion, and finally, in the fluid filling the body cavity.

40. *Hemoglobin in Turtle Parasites.* G. W. WHARTON, Duke University.

In the cold water extracts of two intestinal parasites of turtles, a trematode *Cercorchis robustus* and a nematode *Camallanus trispinosus*, substances have been found which are thought to be hemoglobin. These materials have an absorption spectrum similar to that of hemoglobin. The effect of oxygen on the absorption bands of both pigments is likewise similar to its effects on the absorption bands of hemoglobin. Hemoglobin has been reported from a nematode (*Ascaris*) before, but this is the first record of hemoglobin in a trematode. The discovery of the presence of hemoglobin in intestinal parasites lends support to the view, now not generally accepted, that parasites of the digestive tract use molecular oxygen for respiration.

41. *A Study of Haemoproteus sacharovi in Pigeons and Mourning Doves with Notes on H. maccallumi of Mourning Doves.* G. ROBERT COATNEY AND EVALINE WEST, U. S. Public Health Service and Peru State Teachers' College.

Natural infections of *Haemoproteus sacharovi* have been studied in 13 young mourning doves (*Zenaidura macroura carolinensis*) from 1 to 66 days and in 6 common pigeons (*Columba livia*) from 6 to 26 days. No constant differences, in morphology or rate of growth could be found between the parasites in the two hosts. Young "ring stage" parasites were morphologically mature gametocytes on the 4th day. This seems to be the first record of a natural infection of *H. sacharovi* in the pigeon. Natural infections of *H. maccallumi* were studied in 4 young mourning doves from 21 to 38 days. "Ring stage" parasites were morphologically mature gametocytes on the 7th day; the sex could be determined on the 5th day.

42. *Invasion of Young Red Blood Cells by Merozoites of Human and Avian Plasmodium.* ROBERT HEGNER, Johns Hopkins University.

Preparations are exhibited of blood films stained with brilliant cresyl blue followed by Giemsa. (1) Blood from canaries infected with *Plasmodium cathemerium* contains many young red cells that have been invaded by parasites and old red cells which are free from parasites. (2) Blood from a human being infected with *Plasmodium vivax* contains many infected reticulocytes and many non-infected mature erythrocytes.

43. *Exoerythrocytic Stages in the Development of Plasmodium circumflexum, and a Comparison of these Stages with Toxoplasma.* REGINALD D. MANWELL AND FREDERICK GOLDSTEIN, Syracuse University.

Recent studies of four species of avian malaria (*gallinaceum*, *praecox*, *cathemerium*, *elongatum*) by various workers have apparently shown that in these species exoerythrocytic stages occur in the endothelial and reticulo-endothelial cells in addition to the long recognized cycle in the red cells. The present authors have accordingly examined other species from this point of view, and have been able to demonstrate that similar exoerythrocytic stages occur in at least two strains of *Plasmodium circumflexum*, of different geographical and host origin. These stages are found (as with the first three of the species named above) most abundantly in the lungs, but they also occur in the spleen, liver, bone marrow, heart muscle, and brain. They consist of unpigmented forms which undergo schizogony, frequently producing very large numbers of merozoites (as many as 170 have been observed from a single schizont). The number of merozoites seems to depend chiefly on the size of the host cell. In the brain the endothelial cells of the capillaries are parasitized; in other organs the large monocytes and fixed phagocytic cells are most often invaded. The stages seen in this species are very similar to those described for other species by other investigators (except for *elongatum*), and are also strikingly like those said to occur in the asexual reproduction of *Haemoproteus*. They have so far not been found by the present authors in chronic cases, nor in all active cases. They seem most likely to be abundant in serious infections. Although these stages strongly resemble corresponding stages in the development of *Toxoplasma* in many respects (as already recognized by others), they differ in the fact that the latter seems always to reproduce by binary fission, whereas the former always do so by schizogony. *Toxoplasma* (at least the avian strains observed by the authors) seems also to be difficult to transmit by blood inoculation, and so far no cases have been observed exhibiting parasitization of the endothelial cells of the brain.

44. *Two Types of Toxoplasma-like Bodies in Canaries.* FRUMA WOLFSON, Johns Hopkins University.

Examination of tissues from over 200 canaries infected with eight species of *Plasmodium* belonging to twelve different strains, revealed in the endothelial tissues what are believed to be two distinct type of *Toxoplasma*-like bodies. One of these was referred to by Wolfson (1937) and was later described by Hegner and Wolfson (1938) as a *Toxoplasma*-like parasite. Among the more common stages are the multinucleated schizonts which often occur free in tissues. The number of merozoites in certain stages reaches over 100. With Giemsa stain, the parasites stain deeply, the cytoplasm being blue and the nucleus red. These multinucleate schizonts were present in birds infected with three species of avian *Plasmodium*, (1) the capistrani strain of *P. relictum* (*praecox*), (2) *P. nucleophilum*, and (3) the Wolfson strain of *P. cathemerium*. This type of schizogony occurs in the birds infected with the first two species occasionally. It appears rather constantly in birds infected with *P. cathemerium*. The second type of parasite was usually found within the mononuclear leucocytes. It is generally oval, but sometimes round in shape. It stains very lightly with Giemsa. The cytoplasm is pale blue in color. The nucleus is red, and sometimes consists of several dots of chromatin. This parasite often lies in a notch in the nucleus of the host cell. Other than mononucleate stages were not commonly seen, but sometimes the host cell appeared to contain more than one parasite. This parasite was found associated with all of the eight species of avian *Plasmodium* studied and was observed in at least two canaries and one sparrow which contained no plasmodial infection. On the basis of the material available, the conclusion reached is that at least two types of *Toxoplasma*-like bodies occur in canaries associated with *Plasmodium*.

45. *A Staining Technique for Demonstrating Avian Malaria Parasites in Tissue Sections.* REDGINAL HEWITT, Johns Hopkins University.

A modification of Wolbach's Giemsa tissue stain (1919) has given very good

results in staining tissues of canaries infected with *Plasmodium cathemerium*. After fixation in Zenker-formol and embedding in paraffin, sections of spleen, liver, and bone marrow are cut from 5 to 10 microns thick. The sections are mordanted from 30 minutes to 1 hour in 2.5 per cent potassium bichromate and are then placed in the staining solution for 24 hours. The staining solution is made up as follows:

Distilled water .....	100 cc
0.5% $\text{Na}_2\text{HCO}_3$ .....	2-4 drops
Absolute methyl alcohol .....	3 cc
Giemsa's stain .....	2.5 cc

Differentiation is carried out in 70 per cent alcohol and the slides are dehydrated in xylol-acetone mixtures. Neutral balsam or cedar oil is used as a permanent mounting medium. Mature erythrocytes in blood vessels and sinuses stain orange and parasites within them stain blue. Immature red cells and leucocytes stain in marked contrast to mature erythrocytes. The chromatin of the parasites does not stain red as in blood-film preparations, but all stages of the parasite can be recognized readily. Areas of infarction, hemorrhage, and necrosis are well shown.

46. *Intestinal Helminths Found in Boys Recently Arrived in Washington, D. C., from Various Parts of the United States.* ELOISE B. CRAM AND JOHN P. FOLAN, National Institute of Health, U. S. Public Health Service.

During the past two years approximately 600 boys have been examined for intestinal parasites at the time of their commitment to the National Training School for Boys. The ages of the boys ranged from 11 to 19 years; the group included both whites and negroes. Although the boys came from about 30 states and the District of Columbia, those from the District predominated. The procedure used for the detection of parasites consisted of microscopical examination of (1) NIH anal swabs, principally for pinworms, and (2) fecal specimens, principally for parasites other than pinworms. On approximately one-half of the boys only one anal swab was made. Subsequently the procedure was changed and on the other half of the total number from 3 to 6, usually 4, anal swabs were made, usually on consecutive days. In the single swab series, 8.5 per cent of the boys showed pinworm infections. In the multiple swab series, the incidence of pinworms was approximately twice as great. Hookworms, whipworms, ascarids and tapeworms were also found. Correlations between the incidence of the parasites and the age, race and geographical origin of the boys will be shown on charts.

47. *Morphological, Physiological and Epidemiological Studies on Eimeria bukidnonensis Infection in a Group of Sixty Dairy Heifers.* DONALD W. BAKER, N. Y. State Veterinary College, Cornell University.

Demonstration.

48. *Lucilia sp. Attacking Sheep at Beltsville, Maryland.* PAUL D. HARWOOD AND ARTHUR C. JERSTAD, U. S. Bureau of Animal Industry.

Four lambs, known to be heavily parasitized with helminths, were purchased August 11, 1938, at Sudley, Md., and transferred to experimental pastures at the National Agricultural Research Center at Beltsville, Md. On August 21, 1938, it was noted that two of these lambs had become infested with maggots about the hind quarters. The maggots were identified as those of a species of *Lucilia*, probably *L. sericata*. As the tails of these lambs had never been docked, the hind quarters had become somewhat soiled with feces. The adhering feces probably contained more or less blood because the lambs were infected with blood-sucking nematodes. These feces were, therefore, an attractive bait for blowflies. One sheep was treated with chloroform without effect on the maggots.

However, dusting the affected areas with phenothiazine, after the wool had been clipped, quickly destroyed the maggots but did not prevent re-blowing in the case of one lamb left exposed on the pasture. Although *Lucilia sericata* has occasionally been reported attacking sheep and cattle in this country, it has not yet become the serious pest of livestock that it has proved to be in Australia and England. Some investigators have thought that the parasitic habit has become established in those countries because of the frequency with which the wool of sheep has become soiled with feces and other organic refuse.

49. *Comparative Anatomy of Nemic Excretory and Reproductive Systems.* M. B. CHITWOOD AND B. G. CHITWOOD, U. S. Bureau of Plant Industry, Babylon, N. Y.

Demonstration.

50. *Some Cercariae from Texas Amnicola.* SEWELL H. HOPKINS, Agricultural and Mechanical College of Texas.

A new heterophyid cercaria and the previously described cercariae of *Microcreadium parvum* and *Anallocreadium armatum* will be demonstrated by living material.

51. *Embryology and Life Histories of Some Trematodes of the Genus Plagioporus.* CHARLES G. DOBROVOLNY, University of Michigan.

*Goniobasis livescens* serves as the first intermediate host for four or more species of trematodes of the genus *Plagioporus*. The cercariae of *Plagioporus sinitsini* develop into metacercariae within their sporocysts. The sporocysts with infective metacercariae emerge from the snail and are consumed by various species of fish. In the fish these trematodes migrate to the gall bladder where they develop to sexual maturity in a few weeks. Cotylocercous cercariae of another species emerge from the snail and encyst in *Hyalella knickerbockeri*. Bass and other centrarchids serve as definitive hosts. The life histories of two species of which one encysts in *Chaetogaster diaphanus* and the other appears to encyst in fishes have not been completed experimentally. The development of the excretory and reproductive systems was traced from the cercarial germ ball to the adult worm. A mesodermal primordium gives rise to the excretory bladder which becomes connected with the main excretory trunks. The reproductive organs appear to develop from one genital complex.

52. *Mazocraes cepedianum, a New Monogenetic Trematode from a Freshwater Fish.* HARRY G. KIMPEL, University of Illinois. (Introduced by HARLEY J. VAN CLEAVE.)

Examination of the gills of a number of gizzard-shad from Lake Decatur, Illinois, revealed the presence of a new fresh-water trematode belonging to the sub-order Polyopisthocotylea. The morphological characters of this parasite give evidence that it belongs to the genus *Mazocraes* Hermann, 1872, type genus of the family *Mazocraeidae* Price, 1936. The shape and arrangement of the four pairs of haptoral suckers, the chitinous skeleton of the haptoral suckers, the two pairs of terminal haptoral hooks, and the cirrus hooks serve as critical, diagnostic characters in establishing this as a new species.

53. *Parasite Studies on Ring-necked Pheasants, Phasianus colchicus torquatus (Gmelin) in Minnesota.* O. WILFORD OLSEN, University of Minnesota and Minnesota Conservation Department, St. Paul.

A total of 156 ring-necked pheasants collected in Minnesota have been examined for intestinal parasites. Eighty-four, or 54 per cent, of the birds examined were parasitized with one or more species of parasites. Of these cases, seventy involved only a single species of parasite while fourteen were concerned with multiple infections. Six species of parasites were found. They are dis-

tributed as follows: two roundworms, (1) the cecal worm, *Heterakis gallinae* (Gmelin, 1790) occurred 79 times (50.6 per cent), and (2) the cropworm, *Capillaria contorta* (Creplin, 1838) 4 times (2.6 per cent); (3) one tapeworm, *Chonotaenia infundibulum* (Bloch, 1779) 12 times (7.5 per cent); two echinostome flukes, (4) *Echinoparyphium recurvatum* (Linstow, 1873) twice (1.3 per cent) and (5) *E. contiguum* Barker and Baston, 1915 once (0.65 per cent); and (6) one coccidian, *Eimeria phasiana* Tyzzer, 1929 twice (1.3 per cent). All these parasites have been previously recorded for the pheasant except the two flukes, *Echinoparyphium recurvatum* and *E. contiguum*. The latter has heretofore been known only from the muskrat (*Ondatra zibethica*).

54. *A Hawk Tapeworm Which Produces a Proliferating Cysticercus in Mice.*  
LAWRENCE R. PENNER, University of Michigan Biological Station and University of Minnesota.

A tapeworm of the genus *Cladotaenia* has been found in *Accipiter cooperi* in the Douglas Lake region, Michigan. Eggs fed to laboratory mice on oatmeal pass to the liver and develop both typical and proliferating cysticerci which locate near the periphery of the liver lobes. An invaginated rostellum with a double crown of hooks has developed by the 13th day. No development was observed when eggs were fed to laboratory rats. *Peromyscus leucopus novaborascensis* and *Microtus pinetorum scalopsoides* have been found infected in nature. In a number of naturally infected *Peromyscus* no proliferation was noted. Cysticerci from *Peromyscus* fed to young Cooper's hawks developed into adult worms of the genus *Cladotaenia*. Adult worms did not develop when cysticerci were fed to cats and a screech owl. Proliferating and typical cysticerci from *Microtus pinetorum* did not develop in cats. In *Peromyscus* and *Microtus* the liver, kidneys, lungs, and body cavity were infected. In experimentally infected mice the liver and in one case the lung were infected. *Peromyscus* is believed to be the normal intermediate host of the parasite.

55. *Variability in Hook Measurement in the Acanthocephala.* HARLEY JONES VAN CLEAVE, University of Illinois.

Differences in measurements do not always reflect actual differences in size. In acanthocephalans having a radially symmetrical proboscis, differences in hook measurements may be attributed to three chief groups of factors: personal error, individual variability within the species, and inaccuracy in measurement due to form of the hook and orientation of the hooks on curved surfaces. Unselected measurements have no necessary validity. Orientation and foreshortening of the proboscis as well as of its individual hooks must be considered before comparisons are made and critical measurements are recorded. Specific instances will be discussed.

56. *Larval Trematode Infection in Juveniles of Physa parkeri* Currier. W. W. CORT, Johns Hopkins University, D. B. McMULLEN, University of Oklahoma, and LOUIS OLIVIER, New York University.

*Physa parkeri* in the Douglas Lake region of Michigan was found to live over only one winter. Adults of this species collected early in July from a beach on Douglas Lake measured from 15 to 25 mm in length. By the first of September these adults were all dead, being represented only by empty shells. Juveniles of this same species collected from this same beach late in July varied in length from 3 to 17 mm and those from a collection made about five weeks later from 10 to 21 mm. In the first of these collections 185 out of 293 (63 per cent) of the juvenile snails were infected with larval trematodes belonging to five different species, 165 or 56 per cent of the total collection being infected with the cercaria of *Echinoparyphium recurvatum*. Immature infections with this echinostome were found in four snails 3 and 4 mm in length and mature infections in five snails 5 mm in length. The miracidia must have penetrated into these snails

soon after they had escaped from the egg membrane. In the second collection from this beach 149 out of 440 (34 per cent) of these juvenile *P. parkeri* were infected with larval trematodes, the incidence of the cercaria of *E. recurvatum* being only 19 per cent. It is suggested that this reduction in incidence is due to the injurious effect of this echinostome on the young snails, which produced a higher death rate among those infected.

57. *Host-parasite Relationship of Larval Trematodes in Oligochaete Worms.*  
CHARLES G. DOBROVOLNY, University of Michigan.

*Chaetogaster limnaei* and *C. diaphanus* readily become infected with a species of corynolocercous cercariae. Other oligochaete worms display varying degrees of infectivity with these cercariae. *Aeolosoma* sp. are less susceptible than *Chaetogaster*. *Stylaria* and *Nais* occasionally become infected experimentally. Encystment within *Dero* was never successful, for a few hours after penetration of the cercariae the tissue surrounding the cyst was sloughed off liberating the cyst. Another method of getting rid of the infection was also employed. Frequently these infected worms separated into two or more parts. The separations occurred in the region of the encysted cercariae and always resulted in the extrusion of the cysts from the oligochaete worm.

58. *A New Heterophyid Cercaria from Texas.* SEWELL H. HOPKINS, Agricultural and Mechanical College of Texas.

A previously undescribed monostome cercaria of the Pleurolophocercous type was found in 13 of 1000 snails (*Amnicola peracuta* Pilsbry and Walker) from the Little Brazos River, Brazos County, Texas. This cercaria, evidently belonging to some member of the Heterophyidae, is distinguished by having on the tail one median dorsal fin, two separate ventral fins, and a caudal fin connected to the distal ventral fin by a narrow extension. There are seven pairs of penetration glands but no specialized penetration spines. Pigmented eyespots are present. The excretory bladder is spherical when expanded, with two narrow transverse cornua. The body is covered, at least anteriorly, with minute spines. The body is 0.17 mm long, 0.07-0.08 mm wide, and 0.05 mm deep; the tail is 0.24 mm long and 0.03 mm wide. Cercariae develop in elongate sausage-shaped rediae about 0.8 mm long and 0.15 mm wide, with pharynx 0.03-0.04 mm long, and a rudimentary gut present only in young rediae.

59. *Schistosomatium from the Muskrat, Ondatra zibethica, in Minnesota and Michigan.* LAWRENCE R. PENNER, University of Michigan Biological Station and University of Minnesota.

A schistosome of the genus *Schistosomatium* has been found in the liver, spleen, and blood vessels of 32 out of 330 muskrats examined during March and April, 1938, from the vicinity of Minneapolis, Minnesota, and in one of two muskrats examined at the University of Michigan Biological Station during August, 1938. Mature worms are about the size of a 12-day infection of *Schistosomatium douthitti* (Cort) in laboratory mice. From worms in copula, only 6 or 7 eggs have been found in the uterus of the female and no more than 8 testes have been counted in the male. Until experimental evidence to the contrary is obtained, the worms are tentatively determined as *Schistosomatium douthitti* (Cort), whose normal host is mice, exhibiting certain morphological changes in the muskrat.

60. *A Strigeid of the Genus Neodiplostomum Which Develops in Laboratory Rats from a Diplostomulum Metacercaria in the Muscles of Rana sphenoccephala.* LAWRENCE R. PENNER, University of Michigan Biological Station and University of Minnesota.

Metacercariae of the diplostomulum type found in the hind-leg muscles of *Rana sphenoccephala*, obtained from a supply company at Englewood, Florida, for

the University of Michigan Biological Station, developed into adult strigeids of the genus *Neodiplostomum* when fed to laboratory rats. Adults found in rats examined 2, 3, and 4 weeks after experimental feeding contained only 2 or 3 eggs.

61. *The Life Cycle of a Strigeid Belonging to the Diplostomidae.* LOUIS OLIVIER, New York University.

Tadpoles of *Rana pipiens* were exposed to *Cercaria micradena* Cort and Brackett, 1938, from *Stagnicola palustris elodes* Say of the Douglas Lake region, Michigan. The cercariae readily penetrated the skin of the tadpoles and developed to typical diplostomula with lateral sucking cups, a well-developed holdfast organ, and a hindbody somewhat longer than broad. In all of the tadpoles examined these larvae were localized in the spinal canal, the cavities of the brain, and the spaces enclosed by the meninges. In the position of the metacercariae this species resembles *Tylodelphys excavata* (Rud.) Szidat, 1935. Two infected tadpoles were fed to a domestic pigeon. Upon autopsy 10 days later 123 mature strigeids were recovered from the small intestine of the bird. The specimens were typical diplostomes with lateral sucking cups and a moderately developed holdfast. The average length of thirty specimens measured was 1.15 mm. From preliminary observations the species appears to belong to the genus *Diplostomum*.

62. *The Life Cycle of Stephanostomum tenuum (Linton), Family Acanthocolpidae.* W. E. MARTIN, DePauw University.

Natural infections of *Stephanostomum tenuum* were found in the digestive gland of the snail, *Nassa obsoleta*. The redia possesses a short saccular gut. The ophthalmo-xiphidio cercaria has a simple tail; two approximately equal suckers; a long prepharynx, globular pharynx, short esophagus, and rudimentary gut; four cephalic glands on each side of the body; a weakly Y-shaped excretory bladder that almost reaches to the ventral sucker; a flame cell formula of  $2[3+3+3+3+3+3]$ ; a spinous cuticula in the anterior region of the body and a double ring of about 42 large spines around the oral sucker. Cercariae were experimentally fed to small fishes, *Menidia menidia*, and were found to encyst in the liver. The metacercariae increase to several times the size of the cercaria. The 42 oral spines also increase in size. Infected *Menidia* were experimentally fed to puffers, *Sphoeroides maculatus*, and two weeks later nearly mature *Stephanostomum tenuum* were recovered from the intestine. From descriptions of adult worms having remnants of eyespots, it is believed that the eye-spotted cercaria is generally present in the family. From a number of references in the literature it must be concluded that some fish is the usual second intermediate host. The adult worms are intestinal parasites of marine fishes. (This work was made possible through the use of the laboratory facilities maintained by Purdue University at the Marine Biological Laboratory.)

63. *Studies on the Pre-cercarial Development of Stichorchis subtriquetrus (Trematoda: Paramphistomidae).* HARRY J. BENNETT AND ARTHUR G. HUMES, Louisiana State University.

Thirty-two specimens of *Stichorchis subtriquetrus* (Rudolphi) Näsmark, 1937, were collected from the intestinal ceca of a beaver killed at Gonzales, La., March 29, 1938. The eggs deposited by these worms hatch in about 21 days. The miracidium has an epidermal cell formula of 6:8:4:2 and contains at the time of hatching a well-formed redia. The amphibious snail, *Fossaria parva* serves as an experimental intermediate host. The miracidium penetrates the mantle of the snail, retaining its epidermal cells for at least 48 hours after penetration. At about the end of this period it liberates the redia and degenerates. The redia then migrates to the liver of the snail, exhausting in the process a pair of conspicuous "penetration glands." Daughter rediae are born about 21 days after infection of the snail host. They also possess penetration glands which disappear soon after birth.

64. *The Present Status of the Trematode Family Spirorchidae Stunkard.*  
ELON E. BYRD, University of Georgia.

Since Stunkard (1921) created the family Spirorchidae for the reception of the genera *Spirorchis* MacCallum and *Hapalotrema* Looss no less than 3 subfamilies, 15 genera, and 33 species have been added to the family group. In working over the collection of blood flukes from turtles obtained from Reelfoot Lake in Tennessee it has been found necessary to revise completely the family group in order to establish it as a natural family. In the first place, we have been unable to substantiate the morphology of the genus *Unicaecum* Stunkard and, therefore, cannot accept the subfamily *Unicaecuminae* Mehra. On the other hand, the characters on which the subfamilies *Spirorchinae* Stunkard and *Hapalotreminae* Stunkard are founded are interchangeable, with the result that many of the described genera and species occupy intermediate positions between the subgroups. The presence or absence of the acetabulum, the position of the ovary in relation to the testes, the arrangement of the follicles of the testes, the position of the genital ducts and genital pore, and the presence or absence of the pigmented eyespots in the miracidia no longer seem to be characters of sufficient constancy to warrant the separation of the family into subfamilies. Consequently, we have found it possible to recognize a single subfamily, *Spirorchinae*, and 8 genera, *Hapalotrema* Looss, *Spirorchis* MacCallum, *Hapalorhynchus* Stunkard, *Vasotrema* Stunkard, *Unicaecum* Stunkard, *Neospiorchis* Price, *Amphiorchis* Price, and *Learedius* Price. These genera are held to be distinct and valid with the exception of the genus *Amphiorchis* which is questionable, and comprise 36 species, 9 of which are new to science.

65. *Life History Studies on Psilostomum ondatrae Price and Petasiger nitidus Linton (Trematoda).* PAUL C. BEAVER, Lawrence College.

The cercaria of *Psilostomum ondatrae* Price, 1931, was found in *Helisoma antrosum* collected from several localities on and near Douglas Lake, Michigan. It was identified as *Cercaria thomasi* McMullen, 1938. Through experiments and field observations it was found that the cercariae emerge at night and find their way into the lateral line canal of fish, where they form thin-walled cysts. Metacercariae are infective in from a week to 10 days and when fed to various birds (hawks, chickens, ducks, pigeons, and canaries), develop to maturity in the proventriculus. Maturity is reached on the 6th day. Previously reported field hosts for the adult are the muskrat and California gull. The eggs are undeveloped when deposited and require about 3 weeks for development of miracidia. *Petasiger nitidus* Linton, 1928, is a small echinostome previously reported from the horned grebe (*Colymbus auritus*). No additional field hosts have been discovered and only one laboratory species (the canary) has been infected. The cercaria was found in *Helisoma antrosum* and *H. campanulata* in the region of Douglas Lake. It is a new form of the large-tailed type of echinostome cercaria which, being large and an active swimmer, is eaten by minnows and fry of diverse species. In the fish host the metacercariae are found in thick-walled oval cysts in the pharyngeal and esophageal walls, especially the latter. After 9 days the metacercariae are infective and when fed to canaries become mature in the duodenum on the 10th day.

66. *On the Life Cycle of a Tapeworm, Diphyllobothrium sp., from the Herring Gull, Larus argentatus Pont.* LYELL J. THOMAS University of Illinois.

Proglottids of a tapeworm, *Diphyllobothrium* sp., collected from time to time since 1930 along the shores of inland lakes in the United States and Canada, have been identified as belonging to a tapeworm from the herring gull. Complete adult worms which measure up to 49 cm in length were collected from the young birds. Experiments show that the coracidia develop from eggs within 6 to 14 days depending upon temperature and other factors. Procercooids developed to the infective stage in *Diaptomus* sp. within 17 days. Cysts of plerocercoids from

lake herring, *Leucichthys artedi* Le Sueur, fed to young gulls and a six-weeks old kitten were infective. Retention of the adult worm by the birds lasts less than one month.

67. *Life History of the Cecal Fluke, Postharmostomum gallinum, of Poultry.*  
JOSEPH E. ALICATA, University of Hawaii.

Studies have shown that eggs of *Postharmostomum gallinum* eliminated with feces of infected fowls contain mature ciliated miracidia. Land-snails, *Eulota similaris*, reported by the writer (1938, *Science* 88: 129), serve as intermediate host of this parasite. When ingested by these snails, the parasite eggs hatched in the intestine, and the miracidia, after migrating to the liver, developed into branched sporocysts. Germ-cells in the sporocyst developed directly into cercariae and matured about 60 days after infection. The cercaria possesses a short tail having at its extremity two lateral excretory pores. These pores are the openings of two short ducts originating from the base of a common excretory bladder. Mature cercariae leave the interior and migrate to the pericardial cavity of the same snail, presumably through the renal apertures which open externally at the base of the pallial cavity and internally into the pericardial cavity. If snails harboring mature cercariae are kept in a little water, cercariae often leave the body of the snail entirely and may infect other snails. Young laboratory-raised snails exposed to cercarial infection harbored cercariae in the pericardial cavity 15 hours later; about one month after infection the tail of the cercaria containing the excretory pores becomes pinched off at the base of the excretory bladder, thus leaving a single excretory pore. This stage undoubtedly marks the beginning of the adolescaria stage. Adolescariae fed to young birds reached sexual maturity in about one month. Under natural conditions land-snails, *Subulina octona*, have also been found infected with adolescariae of *P. gallinum*.

68. *The Life Cycle of the Frog Bladder Fluke, Gorgoderina attenuata Staf-ford, 1902 (Trematoda: Gorgoderidae).* JOHN S. RANKIN, JR., Amherst College.

A survey of the amphibian fauna around Amherst, Massachusetts, has revealed a high percentage of infection with *Gorgoderina attenuata*. Tadpoles are heavily infected with metacercariae, the bivalve, *Sphaerium occidentale*, with cercariae. All stages in the life cycle have been produced experimentally. Miracidia are ripe when the eggs are shed into the water and hatch almost immediately. They penetrate the gills of *Sphaerium*, after being swept into the gill chamber through the mollusc's incurrent siphon, and develop into mother sporocysts. A single generation of daughter sporocysts gives rise to large numbers of cystocercous cercariae; tadpoles pick up these cercariae with algae, organic debris, etc. Cercariae penetrate the intestinal wall of the tadpole and encyst in the body cavity, especially around the heart and liver. After ingestion by the definitive host (several species of *Rana* and *Triturus v. viridescens*), developmental stages may be found in the intestine, cloaca, ureters, and kidneys; worms mature in the bladder of the host. Metacercariae have nine testes, three on one side, six on the other. Immature adults show gradual fusion of these nine to two, as found in adult individuals. Such a condition indicates the close relationship to *Gorgodera*, a bladder fluke with nine testes in the adult condition.

69. *Observations on the Life History of Spelotrema nicolli n. sp. (Trematoda: Microphallidae), with the Description of a New Microphallid Cercaria.*  
R. M. CABLE AND A. V. HUNNINEN, Purdue University, Oklahoma City University, and the Marine Biological Laboratory.

Metacercariae occurring in the blue crab, *Callinectes sapidus*, were fed to three young herring gulls and at autopsy numerous adults of a new species, *Spelotrema nicolli*, were recovered from the posterior region of the intestine. Three control birds were negative. The following measurements in millimeters

indicate the chief points of difference between *S. nicolli* and described species: length, 0.54; suckers about equal, diameter, 0.057; distance from center of ventral sucker to posterior end of body, 0.225; diameter of male genital papilla, 0.021; eggs,  $0.019 \times 0.009$ . The excretory formula of the metacercaria is  $2[(2+2)+(2+2)]$ . In the youngest metacercariae, a stylet is present and the ventral sucker is undeveloped, clearly indicating that the cercaria is of the *Ubiquita* type. A new species of cercaria belonging to this group was found in *Nassa obsoleta*. Numerous attempts to induce its penetration of the crab were unsuccessful. This larva, for which the name, *Cercaria nassicola*, is proposed, differs from all described members of the *Ubiquita* group in the arrangement of the cephalic gland ducts and the shape of the stylet. On each side are two large, coarsely granular glands with the duct of one opening dorso-laterally, the other ventro-laterally in the region of the oral sucker. Immediately behind these glands are two smaller, finely granular glands with ducts opening near the tip of the stylet. The stylet is 0.023 mm long and laterally compressed. The results of this study support the validity of the *Microphallidae* as a distinct family.

70. *The Life History of Zygocotyle lunatum*. CHARLES H. WILLEY, New York University.

Encysted metacercariae of *Cercaria poconensis* Willey, 1930, from *Helisoma antrosa* developed into adults of the species *Zygocotyle lunatum* in the cecum in ducks, rats and sheep. Eggs first appeared in feces of ducks in 41 days and in that of rats in 46 to 52 days after a single experimental feeding. They are in the one-cell stage and miracidia hatch in 21 days during the summer months, but require up to 37 days at room temperature during the winter. Fourteen laboratory raised snails have been experimentally infected to date and the first cercariae emerge 35 days after exposure to miracidia. Very young snails, measuring about 2 or 3 mm in diameter of shell, are more susceptible to infection than older snails. A mature sporocyst and numerous daughter rediae in mother rediae have been observed and studied. All metacercariae are infective immediately after encystment, and some, after remaining in dishes of water at room temperature for 5 months, showed occasional movement within the cyst and developed to maturity when fed to rats. Metacercariae exposed to outdoor freezing temperatures as low as  $8^{\circ}$  F for 10 days during March survived and developed normally when fed to rats. Metacercariae are not viable if left out of water for 24 hours at room temperature. Infected ducks and rats are immune to further infection with *Z. lunatum*. Infections from a single experimental feeding still persist after more than a year in ducks.

71. *The Development of Cercaria burti Miller, 1923, in Leeches and Ducks*. C. H. WILLEY AND YALE RABINOWITZ, New York University.

Numerous leeches of the species *Herpobdella punctata* were exposed to specimens of *Cercaria burti* from the snail *Helisoma antrosa* collected in New York City. All became infected. The cercariae penetrated readily and in large numbers. If too many enter, the leech dies within a day or two. *Placobdella parasitica* and *Placobdella rugosa* were also exposed but no infection resulted. In the leech the larvae undergo growth and development and encyst after 35 to 40 days. Leeches containing encysted larvae were fed to domestic white ducks. A duck showing strigeid eggs in the feces was killed 28 days after experimental feeding and yielded 75 adult worms from the small intestine. These are tentatively identified as *Apateomon sphaerocephalus* Brandes, 1888. The worms varied in length from 1.8 mm to 2.5 mm and in width from 0.6 mm to 0.8 mm alive and under a cover glass. The operculate eggs as collected from feces are from 0.10 mm to 0.11 mm in length and from 0.06 mm to 0.07 mm in width. The ducks infected were part of a group of 12 under observation in the laboratory for ten months, being used for other life history work and no evidence of a strigeid infection has appeared in any except those which were fed infected leeches. The

eggs appear in the feces in the one-cell stage and attempts to obtain miracidia are now in progress.

72. *Experimental Studies on Posthodiplostomum minimum (MacCallum, 1921), a Trematode from Herons.* M. S. FERGUSON, Rockefeller Institute for Medical Research, Princeton, N. J. (Introduced by NORMAN R. STOLL.)

*Diplostomum minimum* MacCallum, a strigeid trematode from various herons, is referred to the genus *Posthodiplostomum* Dubois. Morphological study and experimentation show that *Cercaria multicellulata* Miller and *Posthodiplostomulum vancleavei* (formerly *Neascus vancleavei*) are developmental stages of *Posthodiplostomum minimum*. Newly hatched, unfed chicks have been experimentally infected to provide an almost limitless supply of maritae. Eggs, produced in small numbers, develop miracidia after about three weeks. These escape and penetrate *Physa gyrina* and *Physa integra* and produce characteristic strigeid sporocysts and cercariae. The cercariae when liberated from the snail can develop further in at least twenty species of fish, of which blunt-nosed minnows (*Hyborhynchus notatus*), blue gills (*Helioperca macrochira*), and common sunfishes (*Eupomotis gibbosus*) have been most extensively utilized in experiments. Encysted metacercariae advance almost to sexual maturity in the fish host and on entering the body of herons or, as earlier reported, very young chicks, excyst, complete their development and produce eggs in two days' time. The process of excystment in the digestive tract of the chick requires less than 15 minutes. With a pepsin and hydrochloric acid solution (pH 2.5, temperature 37° C) and a mild shaking of the container, excystment is accomplished experimentally in a similar time. The entire life cycle under normal conditions probably requires approximately four months for completion. Since infected snail and fish hosts of this parasite, as well as chicks, are readily available over the country, this trematode life cycle represents especially suitable material for classroom study.

73. *Myxosporidia from Tide Pool Fishes of California.* ELMER R. NOBLE, Santa Barbara State College. (Introduced by HAROLD KIRBY, JR.)

There is, apparently, considerable hybridization among the different species and subspecies of *Gibbonsia*, a common tide pool fish of California. So many intergrades are found that it is often difficult to place them in any described species. These fish are commonly infected with myxosporidia, and a study of the parasites might be used to straighten out their relationships. The gall bladder of *Gibbonsia elegans elegans*, of southern California, is infected with *Leptotheca elegans* Noble and *Sphaeromyxa gibbonsia* n. sp. The gall bladder of *G. metzi*, from the same regions, is also infected with *Sphaeromyxa gibbonsia* but not *L. elegans*. The urinary bladder of *G. metzi*, however, is infected with *Myxoproteus notatus* n. sp. An intergrade between *G. elegans* and *G. metzi* was found to be infected with a small variety of *Leptotheca elegans*. The gall bladder of *Gibbonsia evides*, from central California, is infected with *Ceratomyxa gracilis* Jameson, but this parasite has not been found in southern waters. In close association with *Gibbonsia* is the tide pool blenny *Hypsoblennius*, and the tide pool sculpin *Clinocottus*. These are also heavily infected with myxosporidia, but the parasites are distinct from those of *Gibbonsia*. Thus these fish exhibit a definite host-parasite specificity in spite of their close association and interbreeding. The gall bladder of the blind goby, *Typhlogobius californiensis*, is infected with *Trilospora californica* n. g., n. sp. Spores are triangular in shape, have three polar capsules, and measure 16 microns in greatest breadth.

74. *New Crustacean Parasites from the Atlantic Coast of North America.* A. S. PEARSE, Duke University.

During the summer of 1938 *Cleistosaccus paguri* Lilljeborg was found on *Pagurus pubescens* Kröyer along the shores of Googan's Ledge, Mt. Desert Island, Maine. Two entoniscids belonging to the genus *Cancrion* were found in

pilumnid crabs at Beaufort, N. C., and at Ellerslie, Prince Edward Island, Canada. These have been deposited in the U. S. National Museum.

75. *Protospiruriasis, a New Nematode Disease of Captive Monkeys.* A. O. FOSTER AND C. M. JOHNSON, Gorgas Memorial Laboratory, Panama.

During the past two years, protospiruriasis has been the commonest cause of death among white-face monkeys (*Cebus capucinus*) of the laboratory colony. Although the striking aspect of this situation is that a typically rodent parasite (*Protospirura muricola* Gedoelst) has become adapted to primate hosts, its economic aspects are evident from the fact that at least twenty deaths of experimental monkeys have been ascribed to this condition. In light infections, the parasites are localized in the stomach, although in fatal infections the esophagus and nasopharynx are heavily parasitized. Simple obstruction is probably responsible for much of the damage to the host, yet it is clear from microscopic sections that there is extensive tissue irritation, some deep invasion, and occasionally actual perforation with verminous peritonitis. Secondary bacterial involvement is common. Cockroaches (*Rhynparobia maderae?*) serve as intermediate hosts. The infective larvae are 3.0 mm long by 0.08 mm in diameter and have a characteristic terminal rosette of 10 to 12 papillae. In the body cavity of the cockroach, these larvae are coiled like a watch-spring into a diameter of about 0.4 mm and are contained in discoidal cysts measuring 0.65 mm or more in diameter. The natural rate of infection in cockroaches is very high (96 positives in 101 dissections). The intensity of infection is no less remarkable, since an average of over a hundred cysts per dissection on a day's catch was not unusual. Monkeys become infected through ingesting infected cockroaches, while the latter acquire their infections by ingesting the embryonated eggs of the parasite in the feces of the definitive host.

76. *Age Resistance of Rats Against Trypanosoma lewisi and Trypanosoma cruzi.* J. T. CULBERTSON, M. H. KOLODNY AND C. J. DUCA, College of Physicians and Surgeons, Columbia University.

Rats of nursing age groups are less resistant to infection with *Trypanosoma lewisi* or *T. cruzi* than are older rats, the younger animals suffering more intense and much more frequently fatal infections than the older animals. Likewise, the younger age groups do not respond as well as older animals to vaccination with killed suspensions of trypanosomes (*T. lewisi*), the younger animals seldom being completely protected against infection. The difference in response with age against either infection or vaccination depends largely upon correlated differences in function of the reticulo-endothelial system. Elements of this system have been shown experimentally not to function adequately in the young animals to afford these young an effective level of resistance. For example, rats of the younger more susceptible ages do not manifest the leucocyte and monocyte response characteristic of the older age groups after infection, and the spleens of the younger animals do not reach so high a percentage of the total body weight after infection as those of older infected rats. Furthermore, lower agglutinin antibody titers are attained by young rats than by older rats after comparable vaccination with *T. lewisi* antigen. Finally, the Kupffer cells in young rats have a lower capacity for phagocytic activity than those of older rats, at least upon an inert dye, trypan blue. (This last point has not as yet been studied with trypanosomes.) These considerations indicate that reticulo-endothelial function influences significantly the age resistance demonstrated against both a natural and an induced trypanosome of the rat.

77. *Effects of Number and Age of Worms on Development of Primary and Secondary Hymenolepis diminuta Infections in Rats.* ASA C. CHANDLER, Rice Institute.

Rats were infected with counted cysticercoids from laboratory-infected *Tene-*

*brio.* Growth was extremely slow during the first five days, but rapid thereafter; full size was reached in 18 days. The adult size is in inverse proportion to the number of worms present. Both establishment and growth of secondary worms was markedly influenced by the number of primary worms present. The length of time that the primary infection has existed has no influence on the suppression of secondary infections other than by the size of the primary worms. If the worms of a primary infection are eliminated by anthelmintics, the effect on secondary infections is greatly reduced if not entirely destroyed, and residual worms not expelled by the anthelmintic grow larger when not crowded by their fellows. These experiments suggest that premunition to superinfections is a matter of crowding rather than immunity in the ordinary sense.

78. *Specificity of Artificial Acquired Immunity to Strongyloides ratti.* A. J. SHELDON, School of Medicine, University of North Carolina.

The writer's previous report (1937) of successful artificial active immunization of rats against infection with *Strongyloides ratti* as the result of serial injections of heat-killed larvae, is confirmed. Six rats were immunized by 16 injections of dead larvae at 3-day intervals (4 of 500, 8 of 1,000, and 4 of 2,000 larvae in a saline suspension, totaling 18,000). Following a test infection with 1,000 living larvae, these animals yielded 0.6 per cent development, as compared with 30.5 per cent from a group of 6 previously untreated rats which had been given the same test dose. Utilizing this approach, an attempt was then made to immunize rats against infection with *S. ratti* by serial injections of heat-killed larvae of *S. stercoralis*. Nine rats were given 16 injections of dead larvae at 3-day intervals (4 of 500, 8 of 1,000, and 4 of 2,000 larvae in a saline suspension, totaling 18,000). Following a test infection with 1,000 living larvae of *S. ratti*, these animals showed approximately the same percentage of development, 26.9, as did a similar group of previously untreated animals, 24.7. These results indicate that although *S. ratti* and *S. stercoralis* are closely related and are morphologically and biologically very similar, they are, nevertheless, immunologically different.

79. *Constitutionally Dissimilar Lines of Strongyloides ratti.* GEORGE L. GRAHAM, Rockefeller Institute for Medical Research, Princeton, N. J.

Since serial passage of single, homogonically derived *S. ratti* was reported (Graham, 1935), the similar passage of single *S. ratti* established with larvae of heterogonic origin has been studied. In the heterogonic line the establishment of each new parasitic generation involved the isolation of a single adult female of the bisexual generation for subculture to provide indirectly derived larvae. Unity of maternal ancestry was thus assured in both the parasitic and free-living phases of the life cycle in this line. Technically uniform routine was applied to all procedures involved in comparing these two lines of *S. ratti* in a highly inbred strain of laboratory rats. Over exactly comparable periods, the homogonically derived parasites produced a total of nearly 13,000 progeny, of which 15 per cent were adults of indirect development, and the singly established parasites of the heterogonic line produced over 18,000 offspring, of which 87 per cent were indirect sexual forms. The production of adult progeny of indirect development by the singly established parasites of the heterogonic line at a rate nearly six times that observed in the homogonic line indicates that these pure lines of single *S. ratti* are constitutionally distinct. Inherent factors apparently determine the direction of larval development in *S. ratti*.

80. *Studies on Dietary Deficiencies and Iron Salts in Experimental Canine Hookworm Infections.* G. F. OTTO AND J. W. LANDSBERG, Johns Hopkins University.

Seventeen puppies, in two litters, were used in these experiments. All but one dog in each litter were maintained on a generally deficient diet for six weeks

previous to infection and throughout the course of infection. Throughout this period iron ammonium citrate was given daily to half of these animals as their sole source of iron. One animal in each litter was maintained continuously on our normal stock diet. Each of the seventeen animals was given 900 hookworm by mouth. Neither of the dogs on the normal diet were seriously disturbed by the infection, whereas all the other fifteen died. The blood changes, time of death, and number of worms recovered were essentially the same in the dogs receiving the generally deficient diet and in those receiving the same diet supplemented with iron ammonium citrate. In both cases there were reticulocyte responses in excess of 40 per cent following the development of the hookworm anemia. These data offer only a slight and very questionable suggestion that the addition of iron had any significant influence on the reticulocyte response in these animals.

81. *Three New Nematocides with a Consideration of Factors Governing Nematocidal Efficacy.* B. G. CHITWOOD AND M. B. CHITWOOD, U. S. Bureau of Plant Industry, Babylon, N. Y.

A hydrocarbon, aldehyde, or ketone should be a cholesterol solvent to be an effective nematocide at ordinary temperatures. If it is to be used in soil sterilization, the substance should also have marked penetrating properties into the soil. Substances in aqueous solution or emulsion do not have as effective a mode of dispersal in the soil as do substances developing a high vapor pressure. Under a given set of conditions there is probably an optimum vapor pressure beyond which the substance would be lost entirely. It is thought that such vapors act through their tendency to saturate the adherent moisture of the soil particles and of the organisms; thereafter, dissolving or penetrating the lipoidal (cholesterol and waxy) membranes which ordinarily act as a protective device. On the basis of the above theories, the efficacies of numerous chemicals against the nematode *Ditylenchus dipsaci* have been tested in the soil. Of these mesityl oxide, butyraldehyde, and crotonaldehyde as well as the previously used compounds chloropicrin, carbon disulphide, tetrachlorethylene, ethylene dichloride and carbon tetrachloride were found to be of some value. Under the present conditions, the factors governing nematocidal efficacy are apparently the solubility of cholesterol, soil moisture, vapor pressure or boiling point, and water solubility. (Soil texture and temperature were more or less constant.) The physical constants of the 5 most effective nematocides (chloropicrin, mesityl oxide, butyraldehyde, crotonaldehyde, and ethylene dichloride) tested against *D. dipsaci* fell within the following ranges: solubility in water at 20° C, 1:6 to 1:1150 (best 1:30 to 1:440); boiling point, 75 to 128° C; vapor pressure at 20° C, 8 to 92 mm Hg; solvency of cholesterol at 20° C, roughly 1:10 to 1:35. The significance is being investigated.

82. *Critical Tests with Iso-amyl-ortho-cresol for the Removal of Worms from the Dog.* A. C. JERSTAD, U. S. Bureau of Animal Industry.

Stewart (1937, Cornell Vet. 28: 338) has reported that iso-amyl-ortho-cresol is an effective remedy for the removal of *Dipylidium caninum* from the dog. Since preliminary tests failed to show the specific efficacy for this drug as claimed by Stewart, critical tests were undertaken. Seven dogs harboring *D. caninum* were treated as recommended by Stewart except that treatment was preceded by a 24-hour fast rather than a 12-hour fast. After the fast 0.4 gram of sodium bromide was administered. Thirty minutes later iso-amyl-ortho-cresol at the rate of 0.1 cc per pound of body weight, and one ounce of magnesium sulphate in water were administered. No *Dipylidium* were removed from any dog, although about 180 *D. caninum* were found at autopsy. The seven dogs mentioned and five other dogs treated with iso-amyl-ortho-cresol harbored *Taenia pisiformis*, *Toxocara canis*, *Ancylostoma caninum*, and *Trichuris vulpis*. The greatest efficacy was against *T. canis*, the drug removing three of a total of nine specimens from four animals. No *T. pisiformis* were removed from three infected dogs, and only a fraction of a per cent of the specimens of *Ancylostoma caninum* and *Trichuris*

*vulpis* were removed. It appears that iso-amyl-ortho-cresol has slight anthelmintic value against *T. canis*, but practically none against the other parasites encountered in these tests.

83. *The Effect of Dosage and Interval after Infection on Passive Immunity to the Nematode, Nippostrongylus muris.* MERRITT P. SARLES AND WILLIAM H. TALIAFERRO, University of Chicago.

The manifestations of passive immunity elicited by intraperitoneal or intravenous injection of serum from repeatedly infected, highly immune rats into normal rats appear to be essentially the same as those seen in active immunity, and include retention of a few worms in the skin and larger numbers in the lungs, slight delay in the appearance of eggs in the feces, premature crisis in the egg count curve, greatly decreased egg output, and presence of fewer and smaller worms in the intestine at autopsy. As measured by the total number of eggs passed during the 6th to 14th day of infection, the degree of passive immunity was directly related to the amount of serum injected and inversely related to the interval after infection. It was most marked when the dose of serum was largest and when the serum was injected at the time of infection or the day after, and decreasingly marked with decreasing doses of serum or when injected at 3, 5, or 6 days after infection. Especially interesting was the finding that injection of potent immune serum could affect the sexually mature, egg-laying stage of the parasite in the intestine, as well as the younger developmental stages, and could actually cause the premature cure of the infection through expulsion of worms from the intestine. Smaller doses of such serums and larger doses of less potent serums resulted in temporary inhibition of the infection and actual prolongation of egg production and life of the worms. These results again emphasize the primary role of antibodies in the immunity to *N. muris*.

84. *Rapid Loss of Trichinella Larvae Fed to Immune Rats and Its Bearing on the Mechanism of Immunity.* O. R. MCCOY, University of Rochester.

Rats were made immune to further infection with *Trichinella spiralis* by feeding them by stomach tube four sublethal doses of *Trichinella* larvae over a period of several months. Test doses varying in size from 5,000 to 20,000 larvae were then fed to these rats and to control rats of the same age and sex. The larvae were lost very rapidly from the immune animals, a few appearing in the feces as soon as two hours after feeding. Most of the immune rats developed diarrhea within a few hours and after 8 to 18 hours had passed the majority of the larvae fed. These larvae were alive and were infective when fed to normal rats. Control rats, especially those fed 10,000 larvae or more, passed considerable numbers of larvae in the feces, but the majority were retained and developed in the intestine. The rapid loss of larvae from the intestines of immune rats suggests that possibly some allergic reaction may be instrumental in helping to expel the larvae. Whatever the mechanism of immunity may be, it does not exert a lethal effect on the larvae. No evidence of cellular reaction was observed in the intestinal wall of immune rats killed at hourly intervals after the feeding of *Trichinella* larvae.

85. *A Note on the Cultivation of Taenia taeniaeformis Larvae in vitro.* JAMES H. WILMOTH, New York University.

Larval development of the cat tapeworm (*Taenia taeniaeformis*) begins after ingestion of ova by rats and mice. This larva (*Cysticercus fasciolaris*) is normally localized in the liver of the rodents where it may occur in great numbers. Several months are required in the rodent liver before the larva reaches a stage which will be infective when eaten by its definitive host. In the liver, this worm is relatively free of bacteria, if not entirely so. In this respect such a larva is a suitable subject for studies on oligo-septic and aseptic cultivation in vitro. Furthermore, its size is suitable for convenient manipulation. A number of

such larvae were removed from rat livers and introduced into various solutions. It was found that Tyrode's modification of Ringer-Locke solution was suitable for maintaining the larval tapeworms for a period of 14 days, under oligoseptic conditions. The worms were maintained at 37° C, and the media in which they were immersed was changed at 20-hour intervals. Mendelsohn (1935) described aseptic cultivation of 15-day larvae of *Taenia crassicollis* (= *T. taeniaeformis*). Such minute larvae present a different problem than those utilized in my experiments.

'86.) Development of the Microfilaria of *Dirofilaria scapiceps* (Leidy, 1886) in Mosquitoes of Minnesota. PAUL R. HIGHBY, University of Minnesota.

Microfilariae were found in the blood of a snow-shoe hare, *Lepus americanus phaeonotus* Allen, which was collected at Baudette, Minnesota. They were seen in the gut content of an engorged tick, illustrating a type of xenodiagnosis which may often be useful. Wild mosquitoes were allowed to feed upon the rabbit. On the 12th day after feeding, the infective stages of the filarial worm were seen actively moving within the proboscis of the mosquito, *Aedes canadensis* Theobald and *Aedes excrucians* Walker, and in that of *Aedes fitchii* Felt and Young on the 13th day. Upon autopsy the hare yielded fifteen adult *Dirofilaria scapiceps* = *Filaria scapiceps* Leidy, 1886. Attempts at experimental transfer to rabbits have not yet proved successful.

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## CONSTITUTION OF THE AMERICAN SOCIETY OF PARASITOLOGISTS

(Including revisions of Dec. 30, 1931, Dec. 28, 1932, and Dec. 29, 1933)

**NAME AND OBJECT.** The name of the society is the American Society of Parasitologists.

The object of the society is the association of workers in the field of Parasitology for the presentation and discussion of new or important facts and problems in that science and for the adoption of such measures as will tend to the advancement of parasitological teaching and investigation in this country.

**MEMBERSHIP.** The members of the society shall be of two classes, active and foreign honorary.

Any person interested in parasitology may be a candidate for active membership.

Any foreign scientist who has made eminent contribution to Parasitology may be eligible for honorary membership.

Candidates for membership shall be elected by the Council.

**OFFICERS.** The officers of the society shall be a President and a Vice-President, who shall be elected for one year; a Secretary and a Treasurer, who shall be elected for two years; and members at large of the Council.

The Council shall consist of the President, the Vice-President, the Secretary, the Treasurer, and eight members elected by ballot from the society at large, two for four years, two for three years, two for two years and two for one year. After the first year, two members at large of the Council shall be elected each year to serve four years.

If any vacancy occurs among the officers, the Council is authorized to appoint a member to fill out the unexpired portion of the current year.

The routine business of the society shall be administered by the Council.

Five shall constitute a quorum of the Council.

**DUES.** The dues, to include subscription to the *JOURNAL OF PARASITOLOGY*, shall be four dollars the year for active members, unless changed by vote of the society.

**MEETINGS.** There shall be an annual meeting and such other scientific or business meetings as the Council shall determine.

During the annual meeting a business meeting will be held for the election of officers for the ensuing year and for the transaction of other business.

**AMENDMENT.** On recommendation of a two-thirds vote of the Council, the Constitution may be amended by a two-thirds vote of the members present at any regular business meeting of the Society, provided that at least 30 days' notice has been given to the membership of the proposed amendment.

## BIOLOGICAL ABSTRACTS: A COMMUNICATION

FELLOW PARASITOLOGISTS:

As a member of the Board of Trustees of *Biological Abstracts*, my attention has been directed toward the perfection of plans for *Biological Abstracts* for the coming year. Believing that some may be unfamiliar with this plan, I am enclosing a brief account of its main features. Undoubtedly, many of the group will be interested in Section C which brings together the material on microbiology, protozoology and helminthology.

GEORGE W. HUNTER, III

### A NEW FORM OF BIOLOGICAL ABSTRACTS FOR 1939

The emergency subsidy plan for *Biological Abstracts* which was in effect throughout 1938 will be abandoned at the close of the current year. A new plan for the publication of *Biological Abstracts* beginning with 1939 has been adopted by the Board of Trustees. It provides for the usual monthly issue, but, in addition, this issue will be broken up into five sections and will be sold separately.

The details follow:

1. The price of the complete undivided edition, including the index, for single or multiple copies, will be \$25 to any subscriber, institution, library, industrial group, or individual. In this connection it should be stated that it was with great regret that the Board of Trustees came to the conclusion, after thorough study, that on the new self-supporting basis, it would no longer be possible to offer personal subscriptions to the complete edition at a reduced rate as heretofore.

2. The sections offered are:

Section A—*Abstracts of General Biology* to include General Biology, Biogaphy-History, Bibliography, Evolution, Cytology, Genetics, Biometry, and Ecology; price \$4.

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As in the past, members of Societies contributing \$2 or more per person to *Biological Abstracts* will be entitled to (a) deduct the contribution from a sectional or volume subscription, or (b) receive the complete index.

The subscription prices quoted above are for the United States. Add 50¢ per section to cover foreign sectional subscriptions.

The Societies interested in *Biological Abstracts* are being invited to set up committees to help develop standards and editorial policies in their respective fields.

The success of this new plan, however, depends to a large measure upon the support of biologists and librarians alike. Only as the income increases can the Board and Editorial staff materially augment the coverage by *Biological Abstracts*. In order to print, edit and mail a volume of the current size (including sections)

we require \$49,000 next year. A large number of personal subscriptions will be necessary to meet the budget.

In order to facilitate the plans for 1939, subscription blanks are being distributed throughout the membership of the Societies composing the Union as well as to libraries and institutions generally. It is hoped that a large response may be recorded as soon as possible in the office of the Business Manager, *Biological Abstracts*, University of Pennsylvania, Philadelphia, Pennsylvania.

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